Chapter 14

# *Taraxacum* Genus: Extract Experimental Approaches

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Additional information is available at the end of the chapter

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**Abstract**

This chapter presents factors or considerations to be taken into account when selecting the procedure or method for obtaining extracts and bioactive compounds. The genus *Taraxacum* has proved to have several interesting properties and there are numerous techniques and bioassays used to test the antimicrobial properties of extracts. However, the extraction process is crucial to optimize the final biological outcomes. Extraction procedures that until now have been used are simple and inexpensive, however, we wanted to report a series of studies that group valuable results, which could be useful for future studies, enhancing the research carried out by authors from all over the world and also allowing the interrelated study of this genus.

**Keywords:** extract, antimicrobial activity, *Taraxacum* genus, phytochemical bioassay

## 1. Introduction

*Taraxacum* has been worldwide tested against several bacterial and fungal strains under various extract conditions and bioassays, and we compiled enough published information with the aim of comparing and/or relationship between the various existing methods and their result in the antimicrobial profile.

### 1.1. Antimicrobial bioassay methods used in *Taraxacum* genus

Several different methods have been used for testing antimicrobial activity, the application of which most often depends on the available instrumentation and the training of the investigators [1]. Screening for antibacterial and antifungal activity is often done by agar disc diffusion, agar well test diffusion, and agar dilution or microdilution broth. In agar disc diffusion, a paper disc soaked with the extract is laid on top of an inoculated agar plate and is generally

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *Alternaria alternata* | Broth dilution assay | IC50, MIC, Morphological changes | None | 15 μM | 2.9 μM; 1.0 μM; + | [5] |
| *A. alternata* | Paper disc diffusion method | % Inhibition | None | S, S/2, S/10, S/100 | 16.7–76.2% | [6] |
| *A. carbonarius* (Bainier) Thom | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 4%, 0% | [7] |
| *A. flavus* 0064 | Agar tube dilution | Inhibition growth | Terbinafine 12 mg/mL 100% | 15 mg/mL | 70.80% | [8] |
| *A. fumigatus* 66 | Agar tube dilution | Inhibition growth | Terbinafine 12 mg/mL 100% | 16 mg/mL | 84.80% | [8] |
| *A. hidrophila* (food poisoning patients) | Agar diffusion method | Inhibition zone | Cephalotin 30 μg/mL (20 mm) | 10 mg/mL | No activity | [9] |
| *A. niger* | Broth dilution assay | IC50, MIC, Morphological changes | None | 15 μM | 4.2 μM; 2.8 μM; + | [5] |
| *A. niger* 0198 | Agar tube dilution | Inhibition growth | Terbinafine 12 mg/mL 100% | 17 mg/mL | 37.40% | [8] |
| *A. niger* UPCC 3701 | Agar well diffusion | Inhibition zone, antimicotic index | Canesten (23 mm, 1.3) | 30 μg | No activity | [9] |
| *A. niger* van Thiegem | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 3%, 45% | [7] |
| *A. niger* VKM F-33 | Broth dilution assay | IC50 (50% growth inhibition) | None | 6–10 μM | 1.2–5.6 μM | [10] |
| *A. niger* VKM F-33 | Microtiterd method | IC50 | None | 15.6–250 μg/mL | No activity | [11] |
| *A. flavus* QC 6658 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *A. fumigatus* | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *A. niger* | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *B. cereus* | Agar diffusion method | Inhibition zone | Cephalotin 30 μg/mL (22 mm) | 10 mg/mL | 18 mm | [9] |
| *B. cereus* (spoiled rice) | Agar diffusion method | Inhibition zone | Cephalotin 30 μg/mL (20 mm) | 10 mg/mL | 18 mm | [9] |
| *B. cereus* ATCC 1778 | Broth dilution assay | MIC | Cloramphenicol (0.004 μM) | No information | 2.5 μM | [13] |
| *B. cereus* NCTC 7464 | Broth dilution assay | MIC | None | 2 mg/mL | 500 μg/mL | [14] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *B. cereus* NCTC 7464 | Microtiterd method | MIC | None | 2 mg/mL | 250 μg/mL | [15] |
| *B. cinérea* | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 9%, 38% | [7] |
| *B. cinerea* SGR-1 | Broth dilution assay | IC50 (50% growth inhibition) | None | 6–10 μM | 5.2–5.8 μM | [5] |
| *B. cinerea* SGR-1 | Microtiterd method | IC51 | None | 15.6–250 μg/mL | No activity | [11] |
| *B. sorokiniana* | Broth dilution assay | IC50, MIC, Morphological changes | None | 15 μM | >15 μM; >15 μM; | [10] |
| *B. sorokiniana* 6/10 | Broth dilution assay | IC50 (50% growth inhibition) | None | 6–10 μM | 5.2 μM | [5] |
| *B. sorokiniana* F-1446 | Microtiterd method | IC52 | None | 15.6–250 μg/mL | No activity | [10, 11] |
| *B. subtilis* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | weak activity, not indicated | [16] |
| *B. subtilis* | Agar inoculation | MIC | None | No information | 7.0 mg/mL | [17] |
| *B. subtilis* | Disc diffusion method | Inhibition zone | None | No information | 12.04 mm | [18] |
| *B. subtilis* | Disc diffusion method | Inhibition zone | Tetracyclin 10 μg/disc (23.6 mm) | 10 mg/mL | 5.1–97.9% | [19] |
| *B. subtilis* | Broth dilution | MIC | Tetracyclin (MIC 5.0 μg/mL) | 10 μg/mL | 5.1–97.9% | [19] |
| *B. subtilis* | Agar well diffusion | Inhibition zone | None | 120 μg/mL | 10.0–14.0 mm | [20] |
| *B. subtilis* | Broth dilution assay | MIC | Tetracyclin 100 μg/mL (84%) | 50 mg/mL | 10–54% | [4] |
| *B. subtilis* | Disc diffusion method | Inhibition zone | Tetracyclin 100 μg/mL (22 mm) | 50 mg/mL | 11–19 mm | [4] |
| *B. subtilis* ATCC 1149 | Agar well diffusion | Inhibition zone, antimicotic index | Chloramphenicol (8 mm, 0.3) | 30 μg | 11 mm, 0.1 | [9] |
| *B. subtilis* ATCC 6633 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM | 18 mg/mL | No activity | [8] |
| *B. subtilis* ATCC 6633 | Disc diffusion method | Inhibition zone | Control (8 mm) | 1000–2000 μg/mL | 0–12.5 mm | [21] |
| *B. subtilis* ATCC 6633 | Broth inhibition method | % Inhibition | None | 1000–2000 μg/mL | 5.1–97.9% | [21] |


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| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *B. subtilis* ATCC 6633 | Broth dilution assay | MIC | None | No information | No activity | [22] |
| *B. subtilis* KCTC 1021 | Disc diffusion method | Inhibition zone | Control (8 mm) | 500–2000 μg/mL | 8.5–12.5 mm | [23] |
| *B. subtilis* KCTC 1021 | Broth dilution | % Inhibition | None | 1000–2000 μg/mL | 5.1–97.9% | [23] |
| *B. subtilis* VKM 1053 | Agar diffusion method | Inhibition zone | | 6–10 μM | 0.8–1.2 μM | [5] |
| *B. cereus* NCTC 7464 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *B. pumilus* (wildtype hand isolate) | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *B. subtilis* NCTC 10400 (NCIMB 8054) | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *C. albicans* | Disc diffusion method | Inhibition zone | No information | No information | No activity | [24] |
| *C. albicans* | Broth dilution assay | MIC | Tetracyclin 100 μg/mL (68%) | 50 mg/mL | 0–70% | [4] |
| *C. albicans* | Disc diffusion method | Inhibition zone | Tetracyclin 100 μg/mL (20 mm) | 50 mg/mL | 14–20 mm | [4] |
| *C. albicans* ATCC 10231 | Paper disc diffusion method | Inhibition zone | Chloramphenicol 30 mcg (27 mm) | 50 μL/disc | No activity | [25] |
| *C. albicans* ATCC 10231 | Agar diffusion method | Inhibition zone | Anfotericin (0.2 mm) | 200 μg/mL | >200 μg/mL | [26] |
| *C. albicans* ATCC 18804 | Broth dilution assay | MIC | Anfotericin B (0.0004 μM) | No information | 0.039 μM | [13] |
| *C. albicans* ATCC 90028 | Agar well diffusion | | Anfotericin B (100 μg/disc) | 40 μg | 3.0 mm | [27] |
| *C. albicans* UPCC 2168 | Agar well diffusion | Inhibition zone, antimicotic index | Canesten (18 mm, 0.3) | 30 μg | 12 mm, 0.2 | [9] |
| *C. glabrata* ATCC 2001 | Agar diffusion method | Inhibition zone | Anfotericin (0.4 mm) | 200 μg/mL | >200 μg/mL | [26] |
| *C. gloesporoides* | Broth dilution assay | IC50, MIC, Morphological changes | | 15 μM | >15 μM; >15 μM;- | [10] |
| *C. jejuni* | Broth dilution assay | Adhesion, cytotoxicity, Antibacterial | 3-sialyllactose (IC50 1.4 mg/mL) | 500 mg/mL | IC50 < 3 mg/mL, <10%, no activity | [28] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *C. jejuni* NCTC 11168 (ATCC 700819) | Broth dilution assay | MIC, IC50, % Inhibition | Ampicillin (IC50 1.61 μg/mL) | 15 μM | No activity | [29] |
| *C. lagenarium* | Direct inoculation | Rates of Inhibition | Control untreated leaves | No information | 1.90 | [30} |
| *C. lagenarium* | Direct inoculation | Rates of Inhibition | Control untreated leaves | No information | 12.80 | [30] |
| *C. neoformans* ATCC 32608 | Broth dilution assay | MIC | Anfotericin B (0.0008 μM) | No information | 0.039 μM | [13] |
| *C. parapsilepsis* ATCC 22019 | Agar diffusion method | Inhibition zone | Anfotericin (0.4 mm) | 200 μg/mL | >200 μg/mL | [26] |
| *C. sativus* (S. Ito and Kurib.) | Paper disc diffusion method | Inhibition zone | Mancozeb, Thiram, Carboxin, Benomyl (1 mg/disc) | 5 mg/disc | weak activity, not indicated | [31] |
| *C. tropicalis* ATCC 750 | Agar diffusion method | Inhibition zone | Anfotericin (0.4 mm) | 200 μg/mL | 2.0 mm | [26] |
| *C. utilis* ATCC 22023 | Agar diffusion method | Inhibition zone | Anfotericin (0.4 mm) | 200 μg/mL | >200 μg/mL | [26] |
| *C. violaceum* ATCC 12472 | Quorum sensing | Inhibition zone | None | No information | 7 mm | [32] |
| *C. violaceum* ATCC 31532 | Quorum sensing | Inhibition zone | None | No information | No activity | [33] |
| *C. violaceum* NTCT 13274 | Quorum sensing | Inhibition zone | None | No information | No activity | [33] |
| *C. albicans* | Disk diffusion method | Inhibition zone | Ciprofloxacin (5 μg/disc) | 130–200 mg/mL | >200 mg/mL | [12] |
| *C. glabrata* ATCC 2001 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *C. krusei* ATCC 6258 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *C. parapsilosis* ATCC 22019 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *C. michiganense* subesp. *Michiganense* Ac-1144 | Agar diffusion method | Inhibition zone | | 6–10 μM | 0.8–1.4 | [5] |
| *Cupriavidus* sp. | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *E. coccus* ATCC 13048 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM | 19 mg/mL | No activity | [8] |
| *E. coli* | Disc diffusion, broth dilution | Inhibition zone, MIC | Erythromicin (MIC 27 μg/mL) | 10–500 μg/mL | 13.3 mm, MIC 50 μg/mL | [34] |
| *E. coli* | Agar inoculation | MIC | None | No information | 1.0 mg/mL | [17] |
| *E. coli* | Agar diffusion method | Inhibition zone | None | 0.1–1.0 mg/mL | >0.5 mg/mL (1–4 mm) | [35] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *E. coli* | Agar diffusion method | Inhibition zone | Cloramphenicol 10 mg/mL (30.5 mm) | 50–200 mg/mL | 5.25–23.5 mm | [36] |
| *E. coli* | Diet | CFU count | Control | No information | Inhibition | [8] |
| *E. coli* | Disc diffusion method | Inhibition zone | None | 1 g/mL | 10.2–18.5 mm | [37] |
| *E. coli* | Disc diffusion method | Inhibition zone | None | No information | 13.21 mm | [18] |
| *E. coli* | Disc diffusion method | Inhibition zone | Gentamycin 10 μg/disc (18.9 mm) | 10 mg/mL | 12.05–14.21 mm | [19] |
| *E. coli* | Broth dilution | MIC | Gentamycin (MIC 1.25 μg/mL) | 10 μg/mL | 250–500 μg/mL | [19] |
| *E. coli* | Disc diffusion method | Inhibition zone | No information | No information | 11–13 mm | [24] |
| *E. coli* | Agar well diffusion | Inhibition zone | None | 120 μg/mL | 2.0–3.0 mm | [20] |
| *E. coli* | Broth dilution assay | MIC | Tetracyclin 100 μg/mL (78%) | 50 mg/mL | 14–62% | [4] |
| *E. coli* | Disc diffusion method | Inhibition zone | Tetracyclin 100 μg/mL (18 mm) | 50 mg/mL | 12–15 mm | [4] |
| *E. coli* 7075 | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16] |
| *E. coli* 8739 | Agar diffusion method | Inhibition zone | None | 1 mg/mL | >1 mg/mL | [38] |
| *E. coli* ATCC 11229 | Disc diffusion method | Inhibition zone | Control (8 mm) | 500–2000 μg/mL | 11–13.5 mm | [21] |
| *E. coli* ATCC 11229 | Broth inhibition method | % Inhibition | None | 500–2000 μg/mL | 98.1–100% | [21] |
| *E. coli* ATCC 1229 | Broth dilution assay | MIC | None | No information | No activity | [22] |
| *E. coli* ATCC 15224 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM | 20 mg/mL | No activity | [8] |
| *E. coli* ATCC 25322 | Agar well diffusion | Inhibition zone | Gentamycin | 240 mg/mL | 6.5 mm | [39] |
| *E. coli* ATCC 8677 | Paper disc diffusion method | Inhibition zone | Ticarcillin 75 mcg (27 mm) | 50 μL/disc | No activity | [26] |
| *E. coli* ATCC 8739 | Broth dilution assay | MIC | None | S, S/2 | Inhibition | [40] |
| *E. coli* DSM 1103 | Broth dilution assay | MIC | None | 2 mg/mL | No activity | [14] |
| *E. coli* DSM 1103 | Microtiterd method | MIC | None | 2 mg/mL | No activity | [15] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *E. coli* KCTC 2441 | Disc diffusion method | Inhibition zone | Control (8 mm) | 500–2000 μg/mL | 9.0–12 mm | [23] |
| *E. coli* KCTC 2441 | Broth dilution | % Inhibition | None | 1500–2000 μg/mL | 13–98.4% | [23] |
| *E. coli* NCTC 25922 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *E. coli* NCTC 9001 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *E. coli* UPCC 1195 | Agar well diffusion | Inhibition zone, antimicotic index | Chloramphenicol (25 mm, 3.2) | 30 μg | 11 mm, 0.1 | [9] |
| *E. faecalis* | Irrigation in situ | | None | 7 mg/mL | weak activity, not indicated | [41] |
| *E. faecalis* ATCC 19433 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM. Cefixime 1.0 μM | 21 mg/mL | No activity | [8] |
| *E. coli* 0157 NCTC 12900 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *E. coli* DH5 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Enterobacter/Klebsiella* sp. | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *E. faecalis* NCTC 775 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Exophiala* (Wangiella) *dermatitidis* QC 7895 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *F. avenaceum* | Broth dilution assay | IC50, MIC, Morphological changes | | 15 μM | 13.1 μM; 6.7 μM; + | [10] |
| *F. graminearum* VKM F-1668 | Broth dilution assay | IC50 (50% growth inhibition) | | 6–10 μM | >10 μM | [5] |
| *F. oxysporium* Schlecht | Paper disc diffusion method | Inhibition zone | Mancozeb, Thiram, Carboxin, Benomyl (1 mg/disc) | 6 μM | 5.7 μM | [31] |
| *F. oxysporium* TSKHA-4 | Broth dilution assay | IC50 (50% growth inhibiton) | Kanamycin | 6 μM | 5.7 μM | [5] |
| *F. oxysporium* TSKHA-4 | Microtiterd method | IC53 | None | 15.6–250 μg/mL | No activity | [11] |
| *H. pylori* | Paper disc diffusion method | Inhibition zone | Control (8 mm) | No information | 10 mm | [42] |
| H. pylori NCTC 11639 (ATCC 43629) | Broth dilution assay | MIC, IC50, % Inhibition | Gentamicin (IC50 0.081 μg/mL) | 500 mg/mL | 25% | [29] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *K. pneumoniae* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16] |
| *K. pneumoniae* | Agar diffusion method | Inhibition zone | Cloramphenicol 10 mg/mL (26.5 mm) | 50–200 mg/mL | — | [36] |
| *K. pneumoniae* | Disc diffusion method | Inhibition zone | Gentamycin 10 μg/disc (18.9 mm) | 10 mg/mL | 13.24–17.72 mm | [19] |
| *K. pneumoniae* | Broth dilution | MIC | Gentamycin (MIC 5.0 μg/mL) | 10 μg/mL | 125–250 μg/mL | [19] |
| *K. pneumoniae* ATCC 13866 | Broth dilution assay | MIC | Cloramphenicol (0.001 μM) | No information | 0.625 μM | [13] |
| *K. pneumoniae* UC 5 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM. Cefixime 1.0 μM | 22 mg/mL | No activity | [8] |
| *K. aerogenes* NCTC 9528 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *K. pneumoniae* 700,603 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *L. monocytogenes* KCCM 40307 | Disc diffusion method. Broth Inhibition method | Inhibition zone. % Inhibition | Control (8 mm). None | 500–2000 μg/mL | 0–12 mm. 5.1–97.9% | [21] |
| *L. monocytogenes* KCCM 40307 | Disc diffusion method. Broth Inhibition method | Inhibition zone. % Inhibition | Control (8 mm). None | 500–2000 μg/mL | 10.5–12 mm. 27.2–94% | [23] |
| *L. monocytogenes* NCTC 11994 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *M. aureum* 4721 E | Broth dilution | MIC | Streptomycin (IC50 1.14 μg/mL) | 500 μg/mL | >500 μg/mL | [41] |
| *M. bovis* BCG | Broth dilution | MIC | Streptomycin (IC50 1.14 μg/mL) | 500 μg/mL | >500 μg/mL | [43] |
| *M. canis* | Agar well diffusion, agar tube dilution | No information | No information | No information | Inhibition | [44] |
| *M. kristinae* | Agar inoculation | MIC | None | No information | 5.0–7.0 mg/mL | [17] |
| *M. laxa* | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 11%, 5% | [7] |
| *M. luteus* | Agar well diffusion | Inhibition zone | None | 120 μg/mL | 5.0–9.0 mm | [20] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *M. luteus* ATCC 10240 | Agar diffusion method | MIC | Erythromicin 1.0 μM | 23 mg/mL | 1.0 μM | [8] |
| *M. piriformis* | Paper disc diffusion method | % Inhibition | None | S, S/2, S/10, S/100 | 5.3–66.7% | [6] |
| *M. smegmatis* MC2 155 | Broth dilution | MIC | Streptomycin (IC50 1.14 μg/mL) | 120 μg/mL | 5.3–66.7% | [43] |
| *M. tuberulosis* H37RA | Broth dilution method | MIC | Rifampim. Isoniazid. Kanamycin | No information | > 200 μg/mL | [44] |
| MRSA (clinical isolated) | Broth dilution assay. Microtiterd method | MIC | None | 2 mg/mL | 375 μg/mL. 500 μg/mL | [14] |
| MRSA AARM 3696 | Disc diffusion method | Inhibition zone | Gentamicin 0.2 mg/disc (20.1 mm) | 0.5–3.0 mg/disc | 6.8–16.5 mm | [45] |
| *P. acnes* | Broth dilution assay | No information | No information | No information | No information | [46] |
| *P. aeruginosa* | Broth dilution assay | No information | No information | No information | No information | [46] |
| *P. aeruginosa* | Disc diffusion, broth dilution | Inhibition zone, MIC | Erythromicin | 10–500 μg/mL | No activity | [34] |
| *P. aeruginosa* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16] |
| *P. aeruginosa* | Agar diffusion method | Inhibition zone | Cloramphenicol 10 mg/mL (26.0 mm) | 50–200 mg/mL | — | [36] |
| *P. aeruginosa* | Disc diffusion method | Inhibition zone | Gentamycin 10 μg/disc (20.0 mm) | 10 mg/mL | 16.52–19.19 mm | [19] |
| *P. aeruginosa* | Broth dilution | MIC | Gentamycin (MIC 2.5 μg/mL) | 10 μg/mL | 125–250 μg/mL | [19] |
| *P. aeruginosa* | Agar well diffusion | Inhibition zone | None | 120 μg/mL | 8.0–13.0 mm | [20] |
| *P. aeruginosa* ATCC 15442 | Broth dilution assay | MIC | Cloramphenicol (0.015 μM) | No information | 2.5 μM | [13] |
| *P. aeruginosa* ATCC 7221 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM. Cefixime 1.0 μM | 24 mg/mL | No activity | [8] |
| *P. aeruginosa* ATCC 9027 | Broth dilution assay | MIC | None | No information | No activity | [22] |
| *P. aeruginosa* ATCC 9721 | Paper disc diffusion method | Inhibition zone | Ticarcillin 75 mcg (20 mm) | 50 μL/disc | No activity | [25] |
| *P. aeruginosa* UPCC 1244 | Agar well diffusion | Inhibition zone, antimicotic index | Chloramphenicol (23 mm, 2.8) | 30 μg | 11 mm, 0.1 | [9] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *P. betae* | Broth dilution assay | IC50, MIC, Morphological changes | | 15 μM | 10.7 μM; 8.0 μM; + | [10] |
| *P. betae* F-2532 | Microtiterd method | IC54 | None | 15.6–250 μg/mL | No activity | [11] |
| *P. debaryanum* VKM F-1505 | Broth dilution assay | IC50 (50% growth inhibition) | None | 6–10 μM | 2.6 μM | [5] |
| *P. digitatum* (Perss) Sacc | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 12%, 42% | [7] |
| *P. expansum* | Paper disc diffusion method | % Inhibition | None | S, S/2, S/10, S/100 | 15.7–69.7% | [6] |
| *P. expansum* Link. | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | No activity | [7] |
| *P. expansum* Link. ATCC 42710 | Paper disc diffusion method | Inhibition zone | Control (8 mm) | 0.1 g/mL | 12 mm | [47] |
| *P. infestans* | Microtiterd method | IC55 | None | 15.6–250 μg/mL | No activity | [11] |
| *P. infestans* OSV 12 | Direct inoculation (potato disc) | Disease development | None | 1.3–5.3 μM | 1.3–5.2 μM | [5] |
| *P. italicum* Wehmer | Microassay method on slides | ICG, IGTE | Only in vivo assays (Imazalil, Fenhexamid) | 0.75X | 2%, 56% | [7] |
| *P. mirabilis* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16] |
| *P. mirabilis* | Agar diffusion method | Inhibition zone | None | 0.1–1.0 mg/mL | >0.5 mg/mL (4–10 mm) | [35] |
| *P. ovale* | Broth dilution assay | No information | No information | No information | No information | [46] |
| *P. syringeae* VKM B-1546 | Agar diffusion method | Inhibition zone | | 6–10 μM | 1.2–1.3 μM | [5] |
| *P. vulgaris* | Disc diffusion, broth dilution | Inhibition zone, MIC | Erythromicin (MIC 26 μg/mL) | 10–500 μg/mL | 10.1 mm, MIC 100 μg/mL | [34] |
| *P. vulgaris* | Agar inoculation | MIC | None | No information | 5.0 mg/mL | [17] |
| *P. vulgaris* | Disc diffusion method | Inhibition zone | Gentamycin 10 μg/disc (19.5 mm) | 10 mg/mL | 13.38–18.33 mm | [19] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *P. vulgaris* | Broth dilution | MIC | Gentamycin (MIC 2.5 μg/mL) | 10 μg/mL | 250–500 μg/mL | [19] |
| *Penicillium* sp. QC 743275 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Pseudomona sp.* | Disc diffusion method | Inhibition zone | No information | No information | 11–21 mm | [24] |
| *P. aeruginosa* NCTC 1662 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *P. aeruginosa* NCTC 27853 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Pseudomonas* sp. | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *R. solani* 18,619 | Agar tube dilution | Inhibition growth | Terbinafine 12 mg/mL 100% | 25 mg/mL | 77.47% | [8] |
| *R. solani* Kühn | Paper disc diffusion method | Inhibition zone | Mancozeb, Thiram, Carboxin, Benomyl (1 mg/disc) | 5 mg/disc | 4.75–17.63 mm | [31] |
| *S. aureus* NCTC 8178 | Microtiterd method | MIC | None | 2 mg/mL | 500 μg/mL | [15] |
| *S. abony enterica* NCTC 6017 | Broth dilution assay | MIC | None | S, S/2 | Inhibition | [40] |
| *S. agalactiae* | Disc diffusion method | Inhibition zone | None | 1 g/mL | 11.1–19.7 mm | [37] |
| *S. aureus* | Broth dilution assay | No information | No information | No information | No information | [46] |
| *S. aureus* | Disc diffusion, broth dilution | Inhibition zone, MIC | Erythromicin (MIC 33 μg/mL) | 10–500 μg/mL | 12.7 mm, MIC 50 μg/mL | [34] |
| *S. aureus* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16} |
| *S. aureus* | Agar inoculation | MIC | None | No information | 5.0 mg/mL | [17] |
| *S. aureus* | Agar diffusion method | Inhibition zone | None | 0.1–1.0 mg/mL | >0.5 mg/mL (1–4 mm) | [35] |
| *S. aureus* | Agar diffusion method | Inhibition zone | Cloramphenicol 10 mg/mL (35.0 mm) | 100–200 mg/mL | 9.0–10.75 mm | [36] |
| *S. aureus* | Disc diffusion method | Inhibition zone | None | 1 g/mL | 9.7–19.9 mm | [37] |
| *S. aureus* | Disc diffusion method | Inhibition zone | None | No information | 16.15 mm | [18] |
| *S. aureus* | Disc diffusion method | Inhibition zone | Tetracyclin 10 μg/disc (38.8 mm) | 10 mg/mL | 11.22–15.07 mm | [19] |
| *S. aureus* | Broth dilution | MIC | Tetracyclin (MIC 2.5 μg/mL) | 10 μg/mL | 250 μg/mL | [19] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *S. aureus* | Disc diffusion method | Inhibition zone | No information | No information | 11–21 mm | [24] |
| *S. aureus* | Agar well diffusion, agar tube dilution | No information | No information | No information | Inhibition | [44] |
| *S. aureus* | Agar well diffusion | Inhibition zone | None | 120 μg/mL | 4.0–11.0 mm | [20] |
| *S. aureus* | Broth dilution assay | MIC | Tetracyclin 100 μg/mL (75%) | 50 mg/mL | 0–76% | [4] |
| *S. aureus* | Disc diffusion method | Inhibition zone | Tetracyclin 100 μg/mL (17 mm) | 50 mg/mL | 9–18 mm | [4] |
| *S. aureus* (salted white cheese) | Agar diffusion method | Inhibition zone | Cephalotin 30 μg/mL (24 mm) | 10 mg/mL | 16 mm | [9] |
| *S. aureus* ATCC 12600 | Paper disc diffusion method | Inhibition zone | Chloramphenicol 30 mcg (27 mm) | 50 μl/disc | No activity | [25] |
| *S. aureus* ATCC 25922 | Agar well diffusion | Inhibition zone | Penicillin | 320 mg/mL | 10.4 mm | [39] |
| *S. aureus* ATCC 43300 | Disc diffusion method | Inhibition zone | Erythromycin 50 μg/well | 40 μg | 7.5 mm | [27] |
| *S. aureus* ATCC 6530 | Broth dilution assay | MIC | None | No information | No activity | [22] |
| *S. aureus* ATCC 6538 | Broth dilution assay | MIC | Cloramphenicol (0.063 μM) | No information | 5.0 μM | [13] |
| *S. aureus* ATCC 6538 | Broth dilution assay | MIC | None | S, S/2 | No activity | [40] |
| *S. aureus* ATCC 6538 | Agar diffusion method | Inhibition zone, MIC | Erythromicin 1.0 μM. Cefixime 1.0 μM | 26 mg/mL | No activity | [8] |
| *S. aureus* ATCC 6538 | Disc diffusion method | Inhibition zone | Control (8 mm) | 1500–2000 μg/mL | 0–12 mm | [22] |
| *S. aureus* ATCC 6538 | Broth inhibition method | % Inhibition | None | 1500–2000 μg/mL | 5.1–97.9% | [22] |
| *S. aureus* ATCC 6538 | Agar diffusion method | Inhibition zone | None | 1 mg/mL | >1 mg/mL | [38] |
| *S. aureus* KCTC 1916 | Disc diffussion method | Inhibition zone | Control (8 mm) | 500–2000 μg/mL | 8.5–13.5 mm | [24] |
| *S. aureus* KCTC 1916 | Broth dilution | % Inhibition | None | 500–2000 μg/mL | 98.1–100% | [24] |
| *S. aureus* KCTC 3881 | Disc diffussion method | Inhibition zone | Gentamicin 0.2 mg/disc (20.1 mm) | 0.5–3.0 mg/disc | 6.8–16.5 mm | [45] |
| *S. aureus* NCTC 8178 | Broth dilution assay | MIC | None | 2 mg/mL | 375 μg/mL | [14] |

| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *S. aureus* UPCC 1143 | Agar well diffussion | Inhibition zone, antimicotic index | Chloramphenicol (20 mm, 2.3) | 30 μg | No activity | [9] |
| *S. australis* | Broth dilution assay | Growth | None | 10–10,000 ppm | No activity | [46] |
| *S. cerviseae* | Broth dilution assay | MIC | Tetracyclin 100 μg/mL (50%) | 50 mg/mL | 0–64% | [4] |
| *S. cerviseae* | Disc diffussion method | Inhibition zone | Tetracyclin 100 μg/mL 18 mm) | 50 mg/mL | 12–15 mm | [4] |
| *S. dysgalactiae* | Disc diffusion method | Inhibition zone | None | 1 g/mL | 13.8–9.6 mm | [37] |
| *S. enterica sorovar typhimurium* ATCC 13311 | Broth dilution assay | MIC | Cloramphenicol (0.001 μM) | No information | 5.0 μM | [13] |
| *S. enteriditis* | Disc diffussion method | Inhibition zone | No information | No information | No activity | [24] |
| *S. epidermis* KCTC 1917 | Disc diffussion method | Inhibition zone | Gentamicin 0.2 mg/disc (24.4 mm) | 130–200 mg/mL | 7.3–16.7 mm | [45] |
| *S. fiexneri* | Disc diffusion, broth dilution | Inhibition zone, MIC | Erythromicin | 10–500 μg/mL | No activity | [34] |
| *S. haemolyticus* | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | Weak activity, not indicated | [16] |
| *S. marscens* | Agar inoculation | MIC | None | No information | 1.0–5.0 mg/mL | [17] |
| *S. sonnei* ATCC 11060 | Broth dilution assay | MIC | Cloramphenicol (0.001 μM) | No information | 2.5 μM | [13] |
| *S. tiphimurium* SARB 69 | Broth dilution assay | MIC | None | 2 mg/mL | No activity | [14] |
| *S. typhi* | Agar well diffussion, agar tube dilution | No information | No information | No information | Inhibition | [44] |
| *S. typhi* (food poisoning patients) | Agar diffusion method | Inhibition zone | Cephalotin 30 μg/mL (18 mm) | 10 mg/mL | 14 mm | [9] |
| *S. typhi* H. | Agar diffusion method | Inhibition zone | Gentamicyn 1 mg/disc, Tetracyclin 2 mg/disc | 4–12 μg/disc | No activity | [16] |
| *S. typhimurium* Reference collection B-69 | Microtiterd method | MIC | None | 2 mg/mL | No activity | [15] |
| *Salmonella poona* NCTC 4840 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |


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| Strains | Bioassay | Results expresion | Positive control | Active concentration | Main results | Reference |
|---|---|---|---|---|---|---|
| *Scedosporium apiospermum* QC 7870 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Serratia marcescens* | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Serratia/Rahnella* sp. | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Staphylococcus* | Diet | CFU count | Control | No information | Inhibition | [8] |
| *S. aureus* (MRSA) 43,300 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *S. aureus* (MSSA) 25,923 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *S. aureus* NCTC 6571 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *Staphylococcus epidermidis* NCTC 14990 | Disk diffusion method | Inhibition zone | Ciprofloxacin 5 μg/disc | 130–200 mg/mL | >200 mg/mL | [12] |
| *T. longifusus* | Agar well diffusion, agar tube dilution | No information | No information | No information | Inhibition | [45] |
| *T. mentagrophytes* UPCC 4193 | Agar well diffusion | Inhibition zone, antimicotic index | Canesten (55 mm, 4.3) | 30 μg | 12 mm, 0.2 | [9] |
| *V. albo-atrum* | Broth dilution assay | IC50, MIC, Morphological changes | None | 15 μM | >15 μM; >15 μM; – | [10] |
| *V. albo-atrum F-2437* | Microtiterd method | IC56 | None | 15.6–250 μg/mL | No activity | [11] |
| *V. cholera* ATCC 11623 | Agar diffusion method | MIC | Erythromicin 1.0 μM. Cefixime 1.0 μM | 27 mg/mL | 12.5 μM | [8] |
| *V. parahaemolyticus* KCTC 2471 | Disc diffusion method. Broth Inhibition method | Inhibition zone. % Inhibition | Control (8 mm). None. | 500–2000 μg/mL | 9.5–15 mm. 5.1–97.9% | [21] |
| *V. parahaemolyticus* KCTC 2471 | Disc diffusion method. Broth dilution. | Inhibition zone. % Inhibition | Control (8 mm). None. | 500–2000 μg/mL | 9.5 - 15 mm. 84.0–97% | [23] |
| *X. campestris* VKM −608 | Broth dilution assay | IC50 (50% growth inhibition) | | 6–10 μM | 1.0–1.2 μM | [5] |

**Table 1.** Principal types of bioassays carried out to determine the antimicrobial activity of the genus *Taraxacum* and their respective results.

used as a preliminary check for antibacterial activity prior to more detailed studies. In the agar well test diffusion method, the extract is deposited into wells cut into the agar and can be used as a screening method when large numbers of extracts or large numbers of bacterial isolates are to be screened. In the agar dilution method, a known concentration of the extract is mixed with the agar prior to strain inoculation. In some cases, the inoculated plates or tubes are exposed to UV light to screen the presence of light-sensitizing photochemicals. In the broth dilution method, different techniques exist for determining the end-point, such as an optical density measurement or the enumeration of colonies by viable count. Antimicrobial activity can also be analyzed by a spore germination assay in broth or on glass slides. *In situ* antifungal activity can be achieved by electron microscopy techniques such as scanning and transmission, as well as by confocal laser scanning microscopy [2].

Direct tissue inoculation is the least used testing method, probably due to the inherent characteristics of the substrate (fruits, vegetables, etc.) that can affect the final results and the standardized laboratory conditions needed for proper result comparisons. Authors also indicate certain restrictions regarding the use of a specific technique. For instance, diffusion techniques seem to be inadequate for non-polar extracts, although many reports with these techniques have been published. Furthermore, when only a small amount of sample is available, diffusion techniques can be considered more appropriate [3]. The disc diffusion method is quick and easy but has several serious shortcomings, such as false positives and negative results due to poor test substance solubility and diffusion through the semi-solid nutritive medium [1].

The agar diffusion and microdilution broth methods are the two most common techniques for determining the antimicrobial activities of *Taraxacum* extracts, but the results are not always reproducible; factors, such as the volume and concentration of the extract placed on the paper disc and the solvent used, vary considerably between studies. When results are compared, the different sensitivities of the assays make antimicrobial activity highly dependent on the selection of the proper test. For example, aqueous fractions of *T. officinale* showed no activity in the disc diffusion test but moderate toxicity against *E. coli* and *B. subtilis* in the broth dilution test [4]. Considering this issue, a list of the bioassays used for testing *Taraxacum* extracts against every strain identified, including the main results, is presented in **Table 1**.

## 2. *Taraxacum* extracts versus commercial antibiotics

When comparing *Taraxacum* extracts to commercial antibiotics, *C. jejuni* adhesion was controlled by a *Taraxacum* extract with an IC50 value of 2.7 mg/mL, slightly less compared to the 3.4 mg/mL obtained with 3′-sialyllactose [28]. In another study, a *T. officinale* extract showed MIC values of 0.004 mg/mL, similar to chloramphenicol with MIC values of 0.001–0.06 mg/mL but considerably lower than amphotericin B with MIC values of 0.4 –0.8 μg/mL for different Gram positive and Gram negative bacteria, respectively [13]. The MIC value of 1.0 mg/mL for *M. luteus* was similar for a methanolic extract and for erythromycin and cefixime, but considerably lower than the MIC value of 12.5 mg/mL obtained for *V. cholera* [8]. In the same work, the inhibition percentage for *Aspergillu*s spp. and *Rhizoctonia* spp. was 37–84%, relatively lower than terbinafine at 12 mg/mL and 100% inhibition.

Generally, researchers select only one technique for evaluating the antimicrobial performance of *Taraxacum*. Few studies have assessed agar disc diffusion and broth dilution in parallel, even when the limitations and advantages for both bioassays have been already stated, as indicated above. An example of this includes the antibacterial properties of an ethanolic extract of the *T. mongolicum* flower, whose fractions were examined by both bioassays [19]. The authors indicated that at 0.1 mg/disc, inhibition results were relatively lower for the plant extract compared to gentamicin and tetracycline, with values between 7.12 and 19.4 mm for the plant extracts and 18.9–38.8 mm for the antibiotics. However, MIC values of 0.06–0.5 mg/mL were obtained for plant extracts against the tested strains. Antibiotics had much lower MIC values of 3.0–5.0 μg/mL, which reaffirms the fact that different bioassays need to be performed in parallel to accurately evaluate the antimicrobial effectiveness of an extract.

The weak activity that some authors have indicated could be improved by higher concentrations, which are needed to reach quantifiable antimicrobial activity under different conditions and assays. For instance, concentrations of *T. officinale* extracts at 130–500 mg/mL were needed to achieve the effect of amphotericin B at 0.2–0.4 μg/mL against Candida strains [26]. In the cases of mancozeb, carboxin, thiram, and benomyl, only 1 mg/disc was effective in inhibiting the growth of *R. solani*, *F. oxysporum*, and *C. sativus*, while the *Taraxacum* extract needed a concentration of 5 mg/disc to achieve the same effect [31]. For *H. pylori* and *C. jejuni*, growth was inhibited by ampicillin and gentamicin at concentrations of 0.5–5.0 μg/mL, while an extract of 500 mg/mL was needed to achieve this inhibition [29]. Considering the disc assay method, an extract of ethyl acetate at 10 mg/mL showed minor inhibition zones (14–18 mm) against *A. hydrophila*, *S. typhi*, *S. aureus*, *B. cereus*, and *E. coli* as compared to cephalothin at 0.03 mg/mL (18–24 mm) [9]. In this study, inhibition diameters were only 20–25% smaller than those reached by the synthetic antibiotic, but the extract concentration was more than 300 times higher, as well as 100 times higher than what would normally be indicated for an attractive natural antibiotic in a commercial setting. In a similar study, the inhibition zones of chloramphenicol at 0.02 mg/mL (10.7–23.5 mm) against *E. coli* and *S. aureus* were lower compared to an ethanolic extract *of T. officinale* at 200 mg/mL (25–30 mm) [36]. In this case, the extract showed higher activity but its concentration was 10,000 times higher than its respective antibiotic. Moreover, methanolic extracts of *T. officinale* at 50 mg/mL resulted in inhibition similar to tetracycline at 0.1 mg/mL using broth dilution and disc assay methods against *E. coli*, *S. aureus*, and *B. subtilis*, among others; that is, a concentration 500 times greater than the antibiotic was necessary to obtain a similar effect [4].

In several studies, different *Taraxacum* extracts exhibited no activity under the tested conditions. For instance, embedded discs with 50 μL of an ethanolic extract of *T. officinale* were not active compared to controls, such as ticarcillin, at 75 μg/disc, and chloramphenicol, at 30 μg/disc [26]. Another study, using a similar extract at 2.5 mg/disc, was inactive against certain strains, as compared to gentamycin, at 1.0 mg/disc, and tetracycline, at 2.0 mg/disc [16]. Different extracts of *T. officinale* leaves and roots (chloroform, methanol, and water) were not active towards *Mycobacterium* compared to streptomycin at 1.14 μg/mL [43]. An ethanolic extract of *T. phaleratum* was also inactive against the same strain compared to rifampin at 0.005–0.01 μg/mL, isoniazid at 0.05–0.1 μg/mL, and kanamycin at 2.5–5.0 μg/mL [44]. A leaf

and root extract of *T. officinale* at 150–200 mg/mL was inactive against 24 bacterial strains, but ciprofloxacin at 5.0 μg/disc showed high antimicrobial activity [12].

The conclusion of these studies may be misleading if slight dilutions or excessively high concentrations are tested. For example, experiments with quantities higher than 1.0 mg/mL for extracts or 0.1 mg/mL for isolated pure compounds should be avoided, whereas the presence of activity is very interesting when concentrations are below 0.1 μg/mL for extracts, and 0.01 mg/mL for isolated compounds [1]. Even when promising results have been achieved, the extracts have also shown contradictory results and can mislead the actual potential of this plant extract if no further investigation is pursued.

In general, active concentrations of *Taraxacum* extracts that achieve inhibitions similar to the synthetic antibiotics are 100–10,000 times higher, which makes *Taraxacum* extracts unsuitable for pharmaceutical development at the moment. However, this is expected since synthetic antibiotics are pure, concentrated compounds, whereas plant extracts are a mixture of different, dilute compounds that act synergistically or antagonistically. Because this situation is common and a characteristic of plant extracts, some authors indicate the possibility of using antibiotics synergistically with plant extracts to improve the action mechanisms against antibiotic-resistant bacteria. No research regarding the synergistic use of *Taraxacum* genus has yet been performed [47].

At present, only commercial and synthetic antibiotics, such as kanamycin, amphotericin B, terbinafine, chloramphenicol, and cephalothin, among others, have been considered as positive controls for establishing strain sensitivity. Comparisons of *Taraxacum* with natural, commercially available antibiotic compounds (such as propolis and other honey products) have been neglected: only one study, regarding antibacterial agents for dental care, contains a comparison with propolis [41]. The comparison with natural antibiotics, for example, honey, might be more realistic in traditional medicine due to the similar vegetable origin and characteristics. As long as no pure compound extraction or purification of *Taraxacum* extracts can be performed reliably for testing antimicrobial activity, the real potential of the *Taraxacum* genus as a source of natural therapeutic agents cannot be established.

Alternatively, instead of only utilizing a chemical antibiotic or a natural antibiotic, antimicrobial synergistic interactions between plant bioactives and some common antibiotics have been reported. There are many advantages to using antimicrobial compounds from medicinal plants, such as fewer side effects, better patient tolerance, lower expense, acceptance due to long history of use, and renewability [48].

## 3. Expression of results in antimicrobial studies

Regarding the expression of the results, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and inhibition percentage of growth are cited by researchers as the most common measurements of antimicrobial performance. In this sense, there are two primary categories for measuring an antimicrobial agent: bactericidal or bacteriostatic. Bacteriostatic refers to an agent that prevents the growth of bacteria and a bactericidal agent kills bacteria, but a complete separation of these definitions might be further pursued. This difference only applies under strict laboratory conditions and is inconsistent for a

particular agent against all bacteria; indeed, it can be influenced by growth conditions, bacterial density, test duration, and extent of reduction in bacterial numbers. Furthermore, bacteriostatic activity has been defined as an MBC/MIC ratio of 4, but numerous technical problems and other factors can affect the determination of that ratio and may have an important impact on the interpretation of the in *vivo* situation. Although MBC and MIC data may provide information on the potential action of antibacterial agents *in vitro*, it is necessary to combine this information with pharmacokinetic and -dynamic data to provide more meaningful predictions of efficacy *in vivo* [49]. Considering this information, no pharmacokinetic or -dynamic studies have been conducted involving the *Taraxacum* genus to date. The majority of the research (not only as an antimicrobial agent, but also as an important medicinal plant) has been performed from a traditional perspective, based on centuries of oral traditions. Only in recent decades has *Taraxacum* been subjected to a considerable amount of tests, principally due to its anti-inflammatory and anti-carcinogenic properties [50]. The antimicrobial properties of this genus have been widely known, but only very general studies have been performed to date, with information that is difficult to interconnect as the action mechanisms and the specific compounds involved have not yet been elucidated. Nevertheless, all the data gathered here provides a promising case for the advantageous commercial usage of this genus.

Considering this general approach, most of the research regarding *Taraxacum* indicates MIC values and inhibition percentages measured in relation to area (in solid cultures) or optical density (in broth cultures). The MBC values were not identified in the consulted references. An observation was made that the MIC definition sometimes differed between publications, another obstacle for data comparison. Some MIC definitions are: "the lowest concentration of the tested products that inhibited the development of microorganisms" [40]; "the lowest concentration required to show a marked inhibition of mycobacterial growth at 72 h" [43]; "the lowest concentration of the compound to inhibit the growth of microorganisms" [19]; and "the lowest sample concentration at which no pink color appeared" [15]. This indicates that MIC values are relative to each study and is compounded by the fact that the complete procedure (including extraction process and sample manipulation) is not standardized and varies considerably among the authors. Furthermore, due to the different solubilities and stabilities of the various compounds in the solvent and the sensitivity of the antimicrobial activity assay performed, directly comparing MIC values is difficult and sometimes confusing. As further examples, in three different studies, the authors reported MIC values in the 0.05–5.0 mg range for ethanol, methanol, or water extracts against *S. aureus* using broth microdilution or agar diffusion method as bioassays [13, 17, 34]. This meant that only MIC values could be used as a comparison against the positive control under the same conditions and may only be considered as an initial screening for further antimicrobial approaches; it cannot provide a reliable comparison between studies. The MIC/MBC ratio might be an option for making antimicrobial activity more independent of assay conditions if similar extraction conditions and sample manipulation have been performed.

## 4. Scaling up from in vitro to in vivo assays

Scaling up an antimicrobial assay from controlled, *in vitro* conditions to that of natural, *in vivo* conditions can be difficult if no proper considerations are taken. For instance, active concentrations

for *in vitro* conditions frequently cannot be reached *in vivo* because the infecting microorganisms are never exposed to constant concentrations of an antimicrobial agent. Microorganisms *in vivo* are subject to competition from other microorganisms present in the tissue, so decreased microbial activity might be due to this competition rather than directly related to the antimicrobial activity of the plant extract. Moreover, temperature, pH, and humidity are more difficult to control in an *in vivo* system. Another issue to consider is that microorganisms in a microtiter plate are in the form of a suspension, whereas bacteria associated with different illnesses naturally form biofilms (organ and tissue infections, dental plaque, etc.), representing an extra challenge for antimicrobial agents [1]. Until now, only studies regarding fruit and vegetable infections have shown a parallel between in vitro and *in vivo* responses to *Taraxacum* extracts (Chapter 1; see Section 2.2.2), but studies in animal tissues and organs have not yet been performed directly.

## 5. Factors affecting antimicrobial activity of extracts

The following sections are referred and discussed in accordance with the information provided in **Table 2** (see Chapter 1) and **Figure 1**. It should be noted that the impact of the parameters mentioned in these sections, except for solvent selection, on the antimicrobial properties of the *Taraxacum* genus has not yet been studied.

### 5.1. Plant material collection

Scientific criteria should be used in the selection of the sample material. To avoid the use of random criteria, the selection of plants should be made from an ethnopharmacological perspective. All the species tested need to be perfectly described and identified, including location, season, date, and time of day harvested. The use of commercial samples should be limited to cases of standardized extracts or defined phytomedicines [3]. The phytochemical composition of *Taraxacum* (and plants in general) is known to depend on the season in which

| ***Taraxacum* parts mentioned in the text** | **Number of extracts tested** | **Positive antimicrobial activity** | | **Negative antimicrobial activity** | |
|---|---|---|---|---|---|
| Root | 51 | 17 | 11% | 34 | 43% |
| Leaves | 38 | 28 | 18% | 10 | 13% |
| Flower | 13 | 10 | 7% | 3 | 4% |
| Honey | 6 | 4 | 3% | 2 | 3% |
| Herb | 3 | 3 | 2% | 0 | 0% |
| Aerial | 32 | 24 | 16% | 8 | 10% |
| Whole plant | 8 | 0 | 0% | 8 | 10% |
| No information | 81 | 66 | 43% | 15 | 19% |
| **Total** | **232** | **152** | | **80** | |

**Table 2.** Summary of the antimicrobial results regarding *Taraxacum* plant parts tested in main studies.

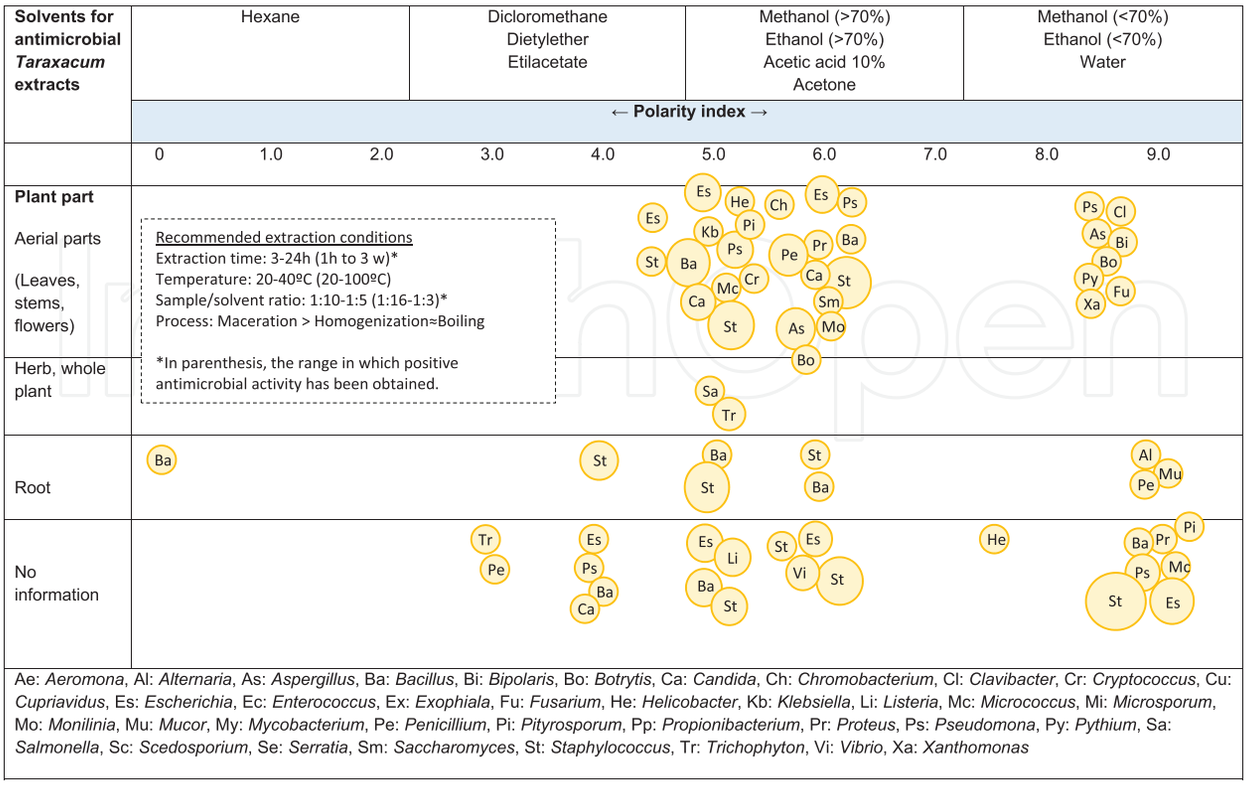


**Figure 1.** Reported extraction conditions to achieve positive antimicrobial results.

it is collected, as well as other ecological and climate factors. For example, sesquiterpene lactones are noticeable in the roots, particularly when harvested in the spring [51]. Sterols, which are present in the leaves throughout the year, are highest during the winter months, whereas levels of sitosterol and cycloartenol esters are highest during periods of sunshine [52]. Few authors indicated in which period of the year the plant was harvested, collected, purchased, or the collection site, another factor that could influence the final concentration of compounds in the extract, even when the same extraction conditions are applied. No *Taraxacum* studies have investigated a possible relationship between harvesting time or collecting site and its antimicrobial properties. Only one study indicated the environmental conditions in which the plant was grown and collected before the antimicrobial assay [27].

## 5.2. Species identification

Generally, there is a lack of taxonomic identification of the species characterized, mentioned occasionally as *Taraxi radix*, *Taraxi folium*, *Taraxi herba*, *Taraxacum* spp., or dandelion, especially when researchers use commercial preparations or purchase the plant from local markets [29, 33, 46]. Samples are commonly obtained in the wild, but the lack of proper identification makes the comparison for antimicrobial properties imprecise for determining the actual efficacy of *Taraxacum* extracts; therefore, only partial conclusions can be pursued and not always extrapolated. For instance, dandelion is used as a common name for several species: khur mang, a name for dandelion in Tibet, can be used for *T. officinale*, *T. mongolicum*, *T. tibetanum*, and *T. Sikkimense* [53]. As previously stated, environmental conditions affect the tissue composition of the plants, but few reports indicate the corresponding information for further

consultation. The importance of proper identification also relates to the risk of toxicity between morphologically similar, but chemically distinct, plants, which is a potential health risk for the communities that harvest medicinal plants in the wild. Only a small portion of the research available mentions proper, expert identification.

### 5.3. Plant part utilization

Reports indicate that compounds present in *Taraxacum* vary within parts of the plant, and even though there are common compounds across sections, these concentrations vary as well [54, 55]. A disadvantage that creates further uncertainties when comparing data is that a considerable amount of studies do not indicate which part of the plant was used. In general, aerial parts (leaves, flowers, and seeds), roots, and whole *Taraxacum* plants have been used in antimicrobial research. Only one *Taraxacum* study indicated differences between a root extract and a leaf extract, in which the root extract was active against *S. aureus* and *S. typhi.* Extracts of plant roots and herbs of different *Taraxacum* species endemic to Turkey displayed significant activity against *M. canis* and *T. longifusus* [44]. Few studies refer to the antimicrobial properties of *Taraxacum* derivatives. *Pseudomona* sp., *S. aureus*, and *E. coli* were inhibited in a disc diffusion assay, but *C. albicans* and *S. enteriditis* were not inhibited by *T. officinale* honey [24]. The pH of dandelion honey is considered the probable antibacterial component observed against *S. aureus* [56]. Analyzing the information gathered in this work (also see **Table 2**), *Taraxacum* root extracts are less effective at fungal and bacterial inhibition than the aerial parts and seem to be more effective on Gram positive than Gram negative bacteria.

### 5.4. Sample manipulation

Several authors propose that plants need to be dried and chopped before extraction. This is a consensus among researches due to the necessity of storing samples prior to processing; however, it is a central issue when testing biological activities because bioactive compounds are highly sensitive and react quickly to changes in environmental conditions. These types of changes are common: a sample is stored at room temperature, refrigerated, frozen, or freeze-dried. In rare cases, further sample manipulation has been reported prior to extraction. Specifically, the removal of lipids and proteins with solvents [31] could also affect the compound profile of the extract and the final antimicrobial activity. In one study, a fresh sample was also homogenized before tested [30]. In our research, sample manipulation seems to be just as adequate whether plant parts are dried under the sun or by oven prior to extraction, or used directly as fresh biomass in extract preparation. Due to the possibility that the material used in the extraction may be contaminated, a white control is considered in the activity bioassays, which is the sample not inoculated with the pathogen, to confirm sterility of the stored sample.

### 5.5. Extraction procedure

Traditional extraction techniques involve solid-liquid extraction with or without high temperatures (maceration, soaking, reflux, etc.), and are characterized by the use of high solvent volumes and long extraction times. These techniques often produce low bioactive extraction yields, low selectivity, and reproducibility can sometimes be compromised. In a common extraction procedure, plant parts are soaked in solvent for extended periods, the slurry is filtered, the filtrate may

be centrifuged multiple times for clarification, and the result may be dried under reduced pressure and re-dissolved in alcohol to a determined concentration. Solid-liquid extractions using soaking, maceration, and homogenization are the most used for *Taraxacum* (although, to a lesser extent, the Soxhlet procedure has been used). Pressurized liquid extraction, subcritical water extraction, and supercritical fluid extraction are presented as novel techniques with important advantages over traditional solvent extraction, such as rapidity, higher yields, and reduced solvent usage. Microwave-assisted extraction and ultrasonic-assisted extraction are pretreatments that can improve the extraction yield by releasing the compounds from the solid matrix [2]. No studies using these techniques have been conducted for the extraction of antibacterial compounds from *Taraxacum* because maceration, blending, and boiling are the most common extraction procedures for this genus. In one study, the sample was sonically treated prior to extraction but no conclusion regarding the effectiveness of this pretreatment can be pursued [22].

### 5.6. Relationship between temperature and extraction time

Temperature directly influences both the solubility equilibrium and mass transfer rate of an extraction process. When temperature is increased, the lower viscosity and surface tension of the solvent improves its diffusion inside the solid matrix, achieving a higher yield and extraction rate along with enhanced diffusivity and solubilization results. The primary disadvantages of applying a higher temperature are increased solvent boil-off and reduced effective contact area between solid and liquid phases. A high temperature can also decrease the cell barrier by weakening the integrity of the cell wall and membrane. Furthermore, bioactive compounds may decompose at high temperatures, which require research on the influence of temperature on the overall yield. Temperatures ranging from cold (4°C), room temperature (20–25°C), and solvent boiling point (50–100°C) have been reported for *Taraxacum*. The majority of the work was conducted in the range of 20–40°C, where the maceration process was proposed and, to a lesser extent, extraction under boiling temperatures has also been indicated (80–100°C, depending on the solvent). Our findings suggest that inhibitory activity is most probable when using a maceration process at mild temperatures (Chapter 1; See **Table 2**).

Determination of the duration of the extraction process required to extract the bioactive compounds, that is, the minimum time at which equilibrium of solvent concentration between inner and outer cells is reached, is important. Most bioactive compounds are sensitive to elevated temperatures and are susceptible to thermal decomposition outside of the original matrix. The extraction time mentioned in literature for *Taraxacum* ranged from 5 min for homogenization, 1–3 hours for boiling, and up to 3 weeks for maceration. A clear relationship between extraction time and antimicrobial activity was not observed in the data presented. However, it is possible that the antimicrobial compounds extracted are relatively stable when extracted by maceration at mild temperatures because numerous positive results regarding inhibitory activity were obtained with this process that included times ranging from 4 hours to 5 days.

### 5.7. Relationship between sample size, solid to solvent ratio, and agitation speed

The particle size of the plant material influences the extraction rate by affecting the total mass transfer area per unit volume, which increases as particle size is reduced. Several authors

| **Polarity index** | **9.0** | **8.2** | **7.7** | **7.4** | **6.3** | **6.3** | **6.2** | **6.0** | **5.9** | **5.6** | **5.5** | **5.4** | **5.1** | **5.1** | **4.4** | **4.1** | **4.0** | **3.1** | **0.0** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genus/solvent** | **Water** | **Ethanol20%** | **Ethanol35%** | **Ethanol40– – 45%** | **Ethanol70–75%** | **Methanol70%** | **Aceticacid** | **Ethanol80%** | **Methanol80%** | **Ethanol90%** | **Methanol90%** | **Ethanol96–100%** | **Methanol100%** | **Acetone** | **Ethylacetate** | **Chloroform** | **Dietylether80%** | **Dichloromethane** | **Hexane** |
| *Aeromona* | | | | | | | | | | | | | | | – | | | | |
| *Alternaria* | | | | | | | + | | | | | | | | | | | | |
| *Aspergillus* | – | | | | | | + | | + | | | | | | | | | – | |
| *Bacillus* | –/+ | | | | + | + | + | + | | | | –/+ | –/+ | + | + | + | | + | –/+ |
| *Bipolaris* | | | | | | | –/+ | | | | | | | | | | | | |
| *Botrytis* | | | | | | | + | | + | | | | | | | | | | |
| *Campylobacter* | | | + | + | | | | | | | | | | | | | | | |
| *Candida* | – | – | | | | + | | | | | | –/+ | + | | | | | + | |
| *Chromobacterium* | – | | | | | | | | | + | | – | | | | | | | |
| *Cochilibus* | + | | | | | | | | | | | | | | | | | | |
| *Colletotrichum* | + | | | | | | – | | | | | | | | | | | | |
| *Cupriavidus* | – | | | | | | | | | | | | | | | | | | |
| *Clavibacter* | | | | | | | + | | | | | | + | | | | | | |
| *Enterobacter* | – | | | | | | | | | | | | – | | | | | | |
| *Enterococcus* | –/+ | | | | | | | | | | | | | | | | | | |
| *Escherichia* | –/+ | + | | – | + | + | | – | + | | – | –/+ | –/+ | + | + | + | | + | – |
| *Exophiala* | – | | | | | | | | | | | | | | | | | | |
| *Fusarium* | – | | | | | | –/+ | | | | | | – | | | | | | |
| *Helicobacter* | | | | + | | | | | | | | + | | | | | | | |
| *Klebsiella* | – | | | | | | | – | | | | | –/+ | | | | | | |

| Polarity index | 9.0 | 8.2 | 7.7 | 7.4 | 6.3 | 6.3 | 6.2 | 6.0 | 5.9 | 5.6 | 5.5 | 5.4 | 5.1 | 5.1 | 4.4 | 4.1 | 4.0 | 3.1 | 0.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genus/solvent** | **Water** | **Ethanol20%** | **Ethanol35%** | **Ethanol40– – 45%** | **Ethanol70–75%** | **Methanol70%** | **Aceticacid** | **Ethanol80%** | **Methanol80%** | **Ethanol90%** | **Methanol90%** | **Ethanol96–100%** | **Methanol100%** | **Acetone** | **Ethylacetate** | **Chloroform** | **Dietylether80%** | **Dichloromethane** | **Hexane** |
| *Listeria* | — | | | | | | | | | | | | + | | | + | | | |
| *Micrococcus* | | | | | | | | | | | | | + | + | | | | | |
| *Microsporum* | | | | | | | | | | | | + | | | | | | | |
| *Monilinia* | | | | | | | | | + | | | | | | | | | | |
| *Mycobacterium* | | | | | — | | | | | | | | — | | | | | | |
| *Mucor* | + | | | | | | | | | | | | | | | | | | |
| *Penicillium* | –/+ | | | | | | | | –/+ | | | | | | | | + | + | |
| *Pityrosporum* | | | | | | | | | | | | + | | | | | | | |
| *Phoma* | + | | | | | | + | | | | | | | | | | | | |
| *Phytophthora* | + | | | | | | | | | | | | | | | | | | |
| *Proteus* | — | | | | + | | | — | | | | | | + | | | | | |
| *Pseudomona* | –/+ | — | | | + | | + | — | | | | –/+ | –/+ | | | + | | + | |
| *Salmonella* | — | | | | | | | | | | | –/+ | | | | | | | — |
| *Saprolegnia* | | | | | | | | | | | | — | | | | | | | |
| *Pythium* | — | | | | | | + | | | | | | + | | | | | | |
| *Rhizoctonia* | + | | | | | | | | | | | | + | | | | | | |
| *Saccharomyces* | | | | | | + | | | | | | | | | | | | | |
| *Salmonella* | | | | — | | | | — | | | | | + | | — | | | | |
| *Scedosporium* | — | | | | | | | | | | | | | | | | | | |

| Polarity index | 9.0 | 8.2 | 7.7 | 7.4 | 6.3 | 6.3 | 6.2 | 6.0 | 5.9 | 5.6 | 5.5 | 5.4 | 5.1 | 5.1 | 4.4 | 4.1 | 4.0 | 3.1 | 0.0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genus/solvent** | **Water** | **Ethanol20%** | **Ethanol35%** | **Ethanol40– – 45%** | **Ethanol70–75%** | **Methanol70%** | **Aceticacid** | **Ethanol80%** | **Methanol80%** | **Ethanol90%** | **Methanol90%** | **Ethanol96–100%** | **Methanol100%** | **Acetone** | **Ethylacetate** | **Chloroform** | **Dietylether80%** | **Dichloromethane** | **Hexane** |
| *Serratia* | — | | | | | | | | | | | | | + | | | | | |
| *Shigella* | — | | | | | | | | | | | | + | | | | | | |
| *Staphylococcus* | −/+ | — | | — | + | + | | −/+ | | | — | −/+ | −/+ | + | + | + | | −/+ | — |
| *Trichophyton* | | | | | | | | | | | | + | | | | | | + | |
| *Vibrio* | | | | | | | — | | | | | | + | | | | | | |
| *Xanthomonas* | | | | | | | + | | | | | | | | | | | | |

(+) Positive antimicrobial activity report. (−) No antimicrobial activity reported. Empty cells indicate no study has been performed so far.

**Table 3.** Antimicrobial activity regarding the solvent tested with *Taraxacum* genus.


http://dx.doi.org/10.5772/intechopen.72849

chopped and ground *Taraxacum* plant material, but few indicate the mesh grain utilized in extract powder selection. Bioactive compounds are dissolved from the solid matrix into the solvent by a physical process under mass transfer principles and compound solubility. When the amount of extraction solvent is increased, the possibility of the bioactive compounds in the solid matrix coming into contact increases. However, the removal of solute from the solvent requires energy. Therefore, if more solvent than needed is used, there will be a higher energy consumption, needlessly increasing processing costs. In the literature reviewed for *Taraxacum*, the sample:solvent ratio ranged between 1:1 and 1:40 w/v. In light of the gathered data, this range has no direct impact on antimicrobial activity but certainly affects the economy of the process. Interestingly, most of the positive results have been achieved with ratios of 1:10–1:4.

A higher agitation speed in solid-liquid extraction is preferred, in accordance with mass transfer theory. In this process, the solute moves from inside the solid to the surface through diffusion or capillary action. Once the compound is on the surface, it is recovered by the solvent through convective mass transfer. Agitation rate affects the mass transfer coefficient (kL) and, at higher rates, improves the convective mass transfer rate, which facilitates the extraction process and leads to increases in extraction yields. For *Taraxacum*, the agitation speed is not usually mentioned in homogenization processes but the most cited value is 170 rpm. Similarly, for the solid: solvent ratio, no direct impact was found in comparisons of different studies.

### 5.8. Solvents

One critical parameter in extraction procedures is the solvent used for sequestering bioactives from the plant matrix. Extractants that solubilize antimicrobial compounds from plants have been ranked by factors such as biohazard risk and ease of solvent removal from fractions. Methanol was ranked second to methylene dichloride and superior to ethanol and water. Even though acetone was rated the highest, it is one of the least used solvents for bioactive extraction. Ethanol and methanol, in contrast, are both commonly used for initial extraction yet may not demonstrate the greatest sensitivity in yielding antimicrobial chemicals on an initial screening [57]. Solvents used for the extraction of bioactive compounds from plants are selected according to polarity and the compounds they are capable of solubilizing. Different solvents may modify results. Apolar solvents (cyclohexane, hexane, toluene, benzene, ether, chloroform, and ethyl acetate) primarily solubilize alkaloids, terpenoids, coumarins, fatty acids, flavonoids, and terpenoids; polar solvents (acetone, acetonitrile, butanol, propanol, ethanol, methanol, and water) primarily extract flavonols, lectins, alkaloids, quassinoids, flavones, polyphenols, tannins, and saponins [58].

The impact of solvent selection is recognized as extremely critical. For example, the gathered data indicate that growth inhibition on fungal strains can be reached by using ethanolic extracts but not aqueous extracts. Moreover, in the same study, inhibition of Gram positive and Gram negative bacteria using an aqueous extract was indicated but no inhibition was achieved using an acetone extract against the same strains [17]. However, it has also been reported that water extracts led to better activity than ethanolic extracts against acne strains, which can be useful in the skin care field [46]. Alcohol extracts tend to display better activity against bacteria and fungi than water extracts, the latter being generally ineffective. Crude *Taraxacum* extracts are commonly used in testing antifungal and antibacterial properties [57], but only a few reports involve the fractioning of the crude sample with other solvents to concentrate and isolate potential

| Solvents used in *Taraxacum* extracts | Number of extracts tested | Positive antimicrobial activity | | Negative antimicrobial activity | |
|---|---|---|---|---|---|
| Low polarity (0–3.0) | 47 | 22 | 9% | 25 | 10% |
| Medium polarity (3.1–6.0) | 100 | 70 | 28% | 30 | 12% |
| High polarity (6.1–9.0) | 101 | 38 | 15% | 63 | 25% |
| **Total** | **248** | **130** | | **118** | |

**Table 4.** Summary of the antimicrobial results regarding the polarity of the *Taraxacum* extracts tested in main studies.

compounds related to microbial activity [4, 15, 19, 22, 24, 42]. These authors agree that antimicrobial activity decreases as follows: ethyl acetate > dichloromethane ≈ chloroform > butanol ≈ hexane > water. This indicates that the antimicrobial compounds should be extracted according to the solvent polarities, showing effective extractions from solvents with a polarity index ranging from approximately 3.0 to 7.0 instead of too polar or apolar solvents. Data analysis indicates that solvents with low (0–3.0) and high (6.1–9.0) polarities are less active against microorganisms than medium polarity solvents (3.1–6.0). A list of the solvents used in research regarding *Taraxacum* antimicrobial activity is presented in **Tables 3** and **4**.

## 6. Perspectives of potential bioassays

As stated above, reports have shown that the antimicrobial potential of different compounds depends not only on the chemical composition of the extract, but also on the targeted microorganism. Further evaluation of the activity of these plants required the study of different conditions. Different parts of the plant (flowers, leaves, stems, etc.), solvent selection (water, alcohol, and organic solvents), extraction procedure (temperature, pH, time, and equipment), bioassay selection (diffusion, dilution, bioautographic methods), and bioassay conditions (volume of inoculum, growth phase, culture medium used, pH of the media, incubation time, and temperature) among others, complicate the comparison of published data.

Studies of the identification and characterization of *Taraxacum* compounds are generally unrelated to a particular pharmacological property. Therefore, the extraction methods for identifying and quantifying extract compounds differ in sample manipulation:temperature, extraction time, and solvent (among others parameters), indicating that comparisons of the extraction methods utilized in antimicrobial activity assays are typically invalid. This complicates the establishment of a relationship between compounds isolated from *Taraxacum* parts and antimicrobial activities.

Nevertheless, *Taraxacum* has been proven effective against most known strains of bacteria, fungi, and protozoa that attack animals and plants through an *in vitro* or *in vivo* approach. All studies of *Taraxacum* extracts against microbes that cause important human diseases (*E. coli*, *S. aureus*, and *A. niger*, among others) were conducted *in vitro*, while microbes causing foodborne diseases with economic implications (*C. lagenarium* for cucumber or *S. australis* for salmonids) were also tested *in vivo*. For humans, only antimicrobial *in vitro* assays were conducted primarily due to the ethical issues of clinical trials. Several authors have mentioned that *Taraxacum*, despite being used as a well-known medicinal plant for centuries, suffers from a lack of *in vivo* evidence and

clinical trials supporting its use [58], which prevents this genus from attracting the possibility of economic development in the pharmacological industry.

Depending on the bioassay selected, diverse extraction conditions should be tested to study the influence of solvents, temperatures, and other parameters that might change outcomes in the extraction process employed. Authors often use non-standardized procedures derived from self-experience combined with bibliographic references, further complicating comparisons between investigations. Even though there are vast amounts of literature on *Taraxacum* biochemical composition and antimicrobial activity, few isolated compounds can be directly related to this activity because studies do not always identify the accurate active fraction and its associated components. In bioassays, the extract generally used is a mixture of compounds; therefore, there is a strong possibility that the activity may be due to the synergy of the compounds present in the extract and not related to a specific compound. The identification, extraction, and isolation of these active compounds are major areas of research that can be initially pursued to formulate a promising source of *Taraxacum* antibiotics. The next step is to test these extracts on *in vitro* and *in vivo* systems to establish pharmacodynamics and interactions, facilitating the commercial attractiveness of *Taraxacum* to the pharmaceutical industry.

The bioavailability, pharmacodynamics, and action mechanisms in *Taraxacum* bioactives have not yet been addressed. Considering that primarily *in vitro* and, to a much lesser extent, *in vivo* studies have been conducted using *Taraxacum* extracts, direct application is the only route that has been considered. If a bioactive compound is going to be suggested as a potential therapeutic agent, other application routes must be tested. Oral ingestion, injection, or inhalation have different characteristics that need to be considered, such as flavor, compound volatility, stability in stomach pH, and possible organ irritation, among others. Therefore, clinical trials are fundamental to evaluating the suitability of *Taraxacum* extract use in pharmacological approaches.

## 7. Conclusion

Only a minor fraction of the *Taraxacum* species has been tested against microorganisms that cause human, animal, and plant diseases. Considering that species can differ in composition due to environmental and genetic characteristics, the evaluated antimicrobial properties could also differ, which means that there is a considerable potential in establishing this genus as a commercial antimicrobial compound. Currently, this genus is considered to have a mild antimicrobial activity compared to other plants, but its worldwide presence and simple cultivation provide an advantage that needs to be assessed more accurately.

Generally, studies do not provide sufficient details concerning the sample manipulation, extraction procedure, or bioassay used, which are necessary for standardization and further statistical comparison. Therefore, despite the published data, it is not possible to conclude which solvent or which conditions provide the optimal results for antimicrobial activity; however, it is possible to set a range of operational parameters that can be used to maximize extract potential.

Isolation and purification of *Taraxacum* compounds needs to be further explored. Although synergy is an important characteristic of plant mixtures responsible for its antimicrobial activity and even though bioactive synthesis is difficult and expensive on a large scale, knowing the

nature of *Taraxacum* extracts and the associated antimicrobial mechanisms may provide important advantages in synthesizing specific structures with improved antimicrobial properties.

Contradictory information is available in the data analyzed; however, these discrepancies are probably the result of different procedures, particular considerations, or inaccurate process descriptions. These differences make it quite possible that the results are not directly related to the full antimicrobial potential of *Taraxacum* but to a limited scope. Therefore, extracts and bioassays must be conducted under a standardized protocol to provide reproducible studies and reliable data comparisons between published articles, which would empower research conducted by authors worldwide and allow for the interrelated study of this genus. In addition, the efficacy of reported biological activity *in vitro* could be validated with *in vivo* assays.

Standardization of the entire procedure (sample manipulation, extraction, and further bioassay) is necessary for comparisons of published data and establishing the exact potential of *Taraxacum*, or any other plant extract, as a commercial antimicrobial agent. The uniformity of an extract is highly susceptible to external factors that influence plant metabolism. This problem could be solved by performing plant breeding techniques with selected *Taraxacum* species grown under controlled environmental conditions.

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