Scientific Research Publishing

American Journal of Plant Sciences, 2026, 17(1), 1-17
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

In Vitro Antibacterial and Antioxidant Activities of Extracts of *Turnera diffusa* Willd. Ex Schult and Its Polyphenol Profile

Rocio Serrano-Parrales[1*], Christian Uriel Aguilera-Ramírez[1], Damaris Nayeli Carrillo-Servin[1], Erick Nolasco-Ontiveros[2], Josué Daniel Bárcenas-Correa[1]

[1]Laboratorio de Farmacobiología, Universidad Nacional Autonoma de Mexico, Tlalnepantla, México
[2]Laboratorio de Bioactividad de Productos Naturales, Facultad de Estudios Superiores-Iztacala, Universidad Nacional Au-Tonoma de Mexico, Tlalnepantla, México
Email: *rocio.serrano@iztacala.unam.mx

How to cite this paper: Serrano-Parrales, R., Aguilera-Ramírez, C.U., Carrillo-Servin, D.N., Nolasco-Ontiveros, E. and Bárcenas-Correa, J.D. (2026) *In Vitro* Antibacterial and Antioxidant Activities of Extracts of *Turnera diffusa* Willd. Ex Schult and Its Polyphenol Profile. *American Journal of Plant Sciences*, **17**, 1-17.
https://doi.org/10.4236/ajps.2026.171001

Received: September 25, 2025
Accepted: January 3, 2026
Published: January 6, 2026

Abstract

Turnera diffusa, belonging to the Turneraceae family, is used by communities in the Tehuacán-Cuicatlán Valley to treat gastrointestinal and respiratory illnesses. The objective of this study was to evaluate antibacterial and antioxidant effects of *T. diffusa*. The plant was collected in Santa María Ixcatlán, within Tehuacán-Cuicatlán Valley (Puebla, Mexico). The antibacterial activity of hexane, acetone, and methanol extracts was evaluated using diffusion and agar dilution methods. Microbial survival curves were generated for susceptible microorganisms. The antioxidant activity was evaluated using the DPPH and ABTS radical scavenging assays and the FRAP ferric reduction assay. The chemical composition was determined using colorimetric reactions, and polyphenol profile was analyzed by reverse-phase HPLC. The acetone extract inhibited the growth of 4 Gram-negative and 3 Gram-positive bacterial strains. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *S. epidermidis* were most susceptible strains (MIC = 2.0 mg/mL). Microbial death curves showed a bactericidal effect of acetone extract on *E. coli* and *S. aureus* after two hours of exposure to the extract (4.0 mg/mL). The methanolic extract exhibited highest antioxidant capacity against DPPH and ABTS radicals (IC_{50} = 45.66 and 116.79 µg/mL, respectively) and the highest ferric reduction capacity (57.08%). This extract also presented highest concentration of total phenols (6.052%), suggesting that this group of secondary metabolites is responsible for effect. The polyphenol profile of these extracts consisted primarily of phenylpropanoids. The results confirm the antibacterial and antioxidant effects, which supports medicinal use of *T. diffusa* in treatment of infectious diseases and oxidative stress.

Keywords

Turnera diffusa, Antibacterial, Antioxidant, Polyphenol Profile

1. Introduction

Infectious diseases represent one of main public health problems worldwide [1], which has been exacerbated by development of antimicrobial resistance in microorganisms, a process accelerated by several factors, primarily the misuse and overuse of antimicrobials [2]. Prolonged administration of antimicrobials often leads to adverse effects, such as gastrointestinal problems, alteration of gut microbiota, and increased oxidative stress [3]. The latter occurs due to excessive production of free radicals, which cannot be counteracted by endogenous antioxidant mechanisms.

Oxidative stress is an underlying factor in chronic and degenerative diseases, such as cardiovascular and neurodegenerative diseases, diabetes, and cancer [4]. This underscores need for research into plant-based alternatives with antioxidant and antimicrobial properties [5]. In Mexico there are around 4500 species of medicinal plants [6], among their main bioactive secondary metabolites are polyphenols, which are attributed biological effects such as antimicrobial [7], antioxidant [8], among others.

Turnera diffusa Willd. Ex Schult., commonly known as "Damiana", is a herbaceous species belonging to Turneraceae family [9]. Polyphenols such as phenolic acids [10], flavonoids, and other compounds with aromatic rings have been reported in this species [11]. In Mexico, it is used to relieve stomach pain, lung diseases related to tobacco abuse, bladder and kidney infections, rheumatism, diabetes, and scorpion stings [12]. Its main use is as an aphrodisiac [13]. In the Tehuacán-Cuicatlán Valley, Puebla (Mexico) it is used against gastrointestinal and respiratory diseases, caused by bacterial strains of genera *Escherichia*, *Klebsiella*, *Salmonella*, *Serratia*, *Pseudomonas* and *Staphylococcus*. The antibacterial effect of *T. diffusa* on species of these genera has been documented by other authors [14] [15].

Previous studies of this species have documented antiaromatase [16], antioxidant [11], cytotoxic [17], gastroprotective [18], hypoglycemic and antidiabetic [19], antimicrobial [20], and hepatoprotective [21] effects, thus demonstrating that *T. diffusa* has high medicinal potential. The objective of this study was to evaluate the antimicrobial and antioxidant effects of extracts of *T. diffusa*, collected in the Tehuacan-Cuicatlan Valley (Mexico), and thereby contribute to the understanding of its medicinal properties, which are not as widely explored given its primary use as an aphrodisiac.

2. Materials and Methods

2.1. Chemicals

Mueller-Hinton agar and broth (Bioxon®) were from Becton Dickinson (BD)

brand. 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, quercetin, 2,2-difenil-1-picrilhidrazil, chloramphenicol, 2,4,6-Tris(2-pyridyl)-s-triazine, potassium persulfate, trolox, iron (III) chloride, sodium acetate, sodium hydroxide anhydrous, gelatin, alpha-naphthol, acetic anhydride, Folin & Ciocalteu's phenol reagent, gallic acid and sodium carbonate used in the experiments were from Sigma-Aldrich brand. Dragendorff reagent Supelco brand and 2,3,5-Triphenyl-tetrazolium chloride solution Merck Millipore brand. Sulfuric, acetic, hydrochloric and phosphoric acids, as well as solvents used (hexane, acetone, methanol, acetonitrile, and water), were analytical grade and HPLC grade from J.T. Baker.

2.2. Plant Material

Turnera diffusa was collected in August 2022, in the municipality of Santa María Ixcatlán, in the state of Oaxaca, Mexico. It is located at 17˚48'90.7" North latitude and 17˚00'61.8" West longitude. The species was identified in the IZTA herbarium of Facultad de Estudios Superiores Iztacala, of the Universidad Nacional Autonoma de Mexico. A voucher specimen (3260 IZTA) was deposited at the herbarium.

2.3. Extracts

The aerial part of *T. diffusa* was dried at room temperature. The extracts were obtained with hexane, acetone and methanol, the extraction was carried out sequentially. 316.7 g of dry and powdered plant material were placed in a flask, 1 L of hexane was added and left to stand for 48 hours, the extract was filtered and concentrated in a rotary evaporator (Heidolph Laborota 4010). Later the same was done with acetone and finally with methanol. The yields obtained from the extracts were: hexane (1.95 g, 0.62%), acetone (7.09 g, 2.24%) and methanol (14.91 g, 4.71%). The extracts were stored at 4˚C and in the dark, for later use in tests.

2.4. Antibacterial Activity

2.4.1. Microorganisms

The bacterial strains used in bioassays were: a) Gram negative bacteria: *Escherichia coli* ATCC 25922, *E. coli* ATCC 53218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhi* ATCC 19430 (donated by the Laboratory of Microbiology Laboratory of Superior Studies Cuautitlan), *Enterobacter aerogenes*, *E. coli* 1249 MR, *E. coli* 182 MR, *E. coli* 28 MR (donated by the FES Iztacala Clinical Analysis Laboratory), *Klebsiella pneumoniae* (isolated from a clinical case and donated by the Angeles Metropolitan Hospital, Mexico). b) Gram positive bacteria: *Staphylococcus aureus* ATCC 12398, *S. aureus* ATCC 29213, *S. aureus* (isolated from a clinical case), *S. aureus* 75 MR, *S. aureus* 83 MR, *S. epidermidis*. These strains were maintained at 4˚C on Mueller-Hinton agar (Bioxon®).

2.4.2. Agar Diffusion Test

The antibacterial activity of extracts was evaluated by Kirby-Bauer method [22].

Inoculums were prepared in 10 mL of Muller-Hinton broth (Bioxon®) and adjusted with 0.5 McFarland standard (10^8 CFU/mL). Inocula were spread on surface of Muller-Hinton agar plates, and triplicate Whatman paper discs of 5 mm diameter, impregnated with 2 mg of extract, were placed on plates. Discs containing 25 µg of chloramphenicol were used as a positive control, and discs containing 10 µL of solvents used (hexane, acetone, and methanol) as negative control. Plates were incubated at 37˚C for 24 h, and inhibition zones were measured and reported in mm.

2.4.3. Agar Dilution Test

The minimum inhibitory concentration (MIC) on susceptible strains was determined by broth dilution method [23]. 100 µL of different concentrations of extracts (0.25 to 3.0 mg/mL), chloramphenicol (1.0 to 20.0 µg/mL), and a control group with 100 µL of Muller-Hinton broth were plated in triplicate in 96-well plates. 100 µL of inocula adjusted to 10^5 CFU/mL were added to each well. Plates were incubated for 24 h at 37˚C and developed with 100 µL/well of tetrazolium chloride (0.1%). MIC was the lowest concentration of extract that visibly inhibited the growth of tested microorganisms.

2.4.4. Bacterial Kinetics Test

The effect of extracts on kinetics of bacterial growth was evaluated by method of Candelaria-Dueñas *et al*., 2021 [24] on strains that showed sensitivity. Different concentrations of extracts were placed in triplicate in tubes with 10 mL of Muller-Hinton broth (Bioxon®) (1.0 to 4.0 mg/mL) and control (without extract). 100 mL of inoculum (10^5 CFU/mL) was added to each tube and placed in incubation at 37˚C. 50 mL samples were taken from each tube at different time intervals (0, 2, 4, 6, 8, 12 and 24 hours), plated on Muller-Hinton agar plates and incubated for 24 hours. CFU/mL were counted to determine the extract concentration and the time required to eliminate or attenuate the growth of microbial population.

2.5. Antioxidant Activity

2.5.1. DPPH Free Radical-Scavenging Activity

The DPPH free radical-scavenging capacity of extract was determined using reagent 1,1-diphenyl-2-picryl hydrazyl and following method of Baliyan *et al*., 2022 [25], with some modifications. Assays were carried out in 96-well plates. 50 µL of extracts at different concentrations (20 to 200 µg/mL for acetone and methanol extracts and 100 to 1000 µg/mL for hexanic extract) and 150 µL of a methanolic DPPH solution (250 µM) were added in triplicate. Plates were incubated in the dark and with constant shaking for 30 min, at 37˚C. Absorbance was measured at 515 nm in an ELISA reader (SLT Spectra ELISA reader). Different concentrations of quercetin (1.5 to 15 µg/mL) were used as a reference standard. Percentage of reduction of the radical was calculated from absorbance data using following formula:

$$\%\ of\ \ antioxidant\ activity = \frac{(Ac - As)}{Ac} \times 100$$

where: Ac = Absorbance of control (DPPH without extract); As = Absorbance of sample (extract or quercetin).

Linear regression model was obtained from the data on reduction percentages of extract and quercetin concentrations, which determined mean inhibitory concentration (IC_{50}), which is concentration that reduces DPPH radical by 50%.

2.5.2. ABTS Free Radical-Scavenging Activity

The antioxidant capacity of extracts on cationic radical ABTS was carried out using the method of Re *et al*., 1999 [26], with some modifications. The radical was generated by an oxidation reaction of ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt 7 mM) with potassium persulfate (2.45 mM). 250 µL of extracts at different concentrations (20 to 200 µg/mL for acetone and methanol extracts and 100 to 1000 µg/mL for hexanic extract) and 2250 µL of ABTS radical (Absorbance = 0.7 ± 0.02 units, at 734 nm) were added in triplicate to test tubes. After 7 minutes of reaction in dark and at room temperature, the absorbance at 734 nm was read (VELAB™ Sspectrophotometer VE-5100UV). Trolox was used as a reference standard at different concentrations (5 to 50 µg/mL). The percentage of reduction of the ABTS radical was calculated with the formula:

$$\%\ of\ \ antioxidant\ activity = \frac{(Ac - As)}{Ac} \times 100$$

where: Ac = Absorbance of control (ABTS without extract); As = Absorbance of sample (extract or Trolox).

The data were graphed, linear regression model was obtained, and median inhibitory concentration (IC_{50}) was calculated, which represents 50% reduction of ABTS radical, which is expressed as milligrams equivalent of Trolox per gram of extract (mgET/g).

2.5.3. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP method was performed using the method of Benzie and Strain, 1999 [27], which measures the ability of antioxidant compounds to donate an electron to Fe^{3+} to form Fe^{2+}. Colorless 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) complex is reduced to colored ferrous complex. The FRAP reagent was prepared by mixing acetate buffer pH 3.6 (300 mM), TPTZ (10 mM) in 40 mM HCl and $FeCl_3$ (20 mM), in a 10:1:1 (v/v/v) ratio. The reagent was kept throughout process in a bath at 37˚C. A standard curve of Trolox was prepared at different concentrations (5 to 50 µg/mL). Samples of 250, 500 and 1000 µg/mL of hexane, acetone and methanol extracts respectively were prepared. In test tubes, 250 µL of Trolox and extract concentrations and 2250 µL of FRAP reagent were added in triplicate. Samples were incubated for 10 minutes at room temperature and in dark, the absorbance was read at 593 nm (VELAB™ Spectrophotometer VE-5100UV). Trolox absorbance data were graphed, linear regression model was obtained, number of equivalent milligrams of Trolox per gram of extract (mgTE/g) and percentage of ferric reduction were calculated.

2.6. Phytochemical Screening of Extracts

The identification of the main groups of secondary metabolites in extracts was carried out by means of reactions that indicate presence of coumarins, phenols, alkaloids, flavonoids, tannins, glycosides, steroids and terpenes. The reagents used were: NaOH-HCl, ferric chloride, Dragendorff, NaOH, gelatin, α-naphthol-HCl and Liebermann-Burchard, respectively [28].

2.7. Total Phenol Content Assay

The total phenol in extracts was determined by Folin-Ciocalteu method [29]. A standard curve was prepared with gallic acid (0.02 - 0.12 mg/mL). Standard solutions of each extract were prepared (0.2 mg/mL). 300 µL of the gallic acid and extract concentrations, 1800 µL of distilled water and 150 µL of the Folin & Ciocalteu's phenol reagent were transferred to test tubes in triplicate. After five minutes of incubation, 450 µL of a Na_2CO_3 solution (200 g/L) were added. The samples were incubated for one hour at room temperature, and absorbance was read at 760 nm (VELABTM Sspectrophotometer VE-5100UV). The gallic acid data were graphed (concentration vs absorbance) and the linear regression model was obtained. The extract absorbance data were used to calculate the milligrams of gallic acid equivalents per gram of extract (mgGAE/g).

2.8. High-Performance Liquid Chromatography

The polyphenol profile of the *T. diffusa* extracts with highest antioxidant activity was determined by high-performance liquid chromatography (HPLC-reverse phase) using a Hewlett-Packard model 1100 with a quaternary pump and diode array detector-DAD. Sample analysis was performed using an Allsphere ODS-1 C18 column (250 × 46 mm, 5 µm). The mobile phase was isocratic [methanol:acetonitrile:water (30:5:65) and 1% phosphoric acid]. Signal analysis of the chromatograms was obtained in the ultraviolet spectrum (254 nm) using Chemstation A.09.03 software.

2.9. Statistical Analysis

The results of tests were analyzed using a one-way ANOVA to determine differences in antibacterial and antioxidant effects between extracts and controls. Linear regression analysis was also performed to determine IC_{50} values of extracts on DPPH and ABTS radicals, as well as mg equivalent concentrations of gallic acid and Trolox, in tests for total phenols and FRAP, respectively. In all cases, results were considered significantly different with a $P < 0.05$. Statistical tests were performed with Microsoft® Excel for Mac software, version 16.91.

3. Results and Discussion

3.1. Antibacterial Activity

The acetone extract of *T. diffusa* showed highest antibacterial activity, inhibiting growth of seven strains (4 Gram-negative and 3 Gram-positive) (**Table 1**). The

largest inhibition zones were observed with chloramphenicol, which was much greater than those of extracts, indicating significant differences between effects of extracts and positive control ($p < 0.01$). However, the results can be considered good, since extracts are complex mixtures in which bioactive compounds are found at variable concentrations, which can be very low or can have antagonistic interactions [30], making it necessary to use high extract concentrations to observe their inhibitory effect on microorganisms.

Regarding acetone extract, largest inhibition zones were observed for *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, and *S. epidermidis* cc (10 ± 1.00 and 13.66 ± 1.15 mm). Relevantly, acetone extract inhibited *P. aeruginosa*, a strain that was resistant to positive control (chloramphenicol). The MIC values of extracts for susceptible strains were 2.0 and 3.0 mg/mL (Table 1). These concentrations are high compared to positive control, which has MIC and MBC values of 1.0 to 12.0 μg/mL. The results of present work coincide with those obtained by other authors, who mention antibacterial effect of *T. diffusa* extracts on *S. aureus*,

Table 1. Antibacterial activity of *T. diffusa* extracts.

Microorganisms		Positive control	Extracts	
			Hexane	Acetone
Gram negative bacteria		**Chloramphenicol**		
E. coli cc	mm	22.33 ± 0.57	na	6.66 ± 0.57*
	MIC	0.004 ± 0.00		2.0 ± 0.00*
K. pneumoniae ATCC 13883	mm	12.33 ± 1.15	na	10.00 ± 1.00
	MIC	0.001 ± 0.00		2.0 ± 0.00*
P. aeruginosa ATCC 27853	mm	na	na	10.00 ± 1.00*
	MIC	na		2.0 ± 0.00
S. marcescens ATCC 14756	mm	20.00 ± 0.00	na	6.33 ± 0.57*
	MIC	0.002 ± 0.00		3.0 ± 0.00*
Gram positive bacteria				
E. faecalis ATCC 14506	mm	11.00 ± 0.00	na	7.66 ± 0.57*
	MIC	0.003 ± 0.00		3.0 ± 0.00*
S. aureus ATCC 29213	mm	20.66 ± 1.15	6.00 ± 0.00*	7.00 ± 0.00*
	MIC	0.008 ± 0.00	3.0	2.0 ± 0.00*
S. aureus cc	mm	19.00 ± 1.00	na	6.00 ± 0.00*
	MIC	0.004 ± 0.00		2.0 ± 0.00*
S. epidermidis cc	mm	26.00 ± 1.00	na	13.66 ± 1.15*
	MIC	0.002 ± 0.00		2.0 ± 0.00*

Halo of inhibition (mm). The extracts were tested with 2 mg of extract/disk. na: no activity was observed. Average values of three repetitions ± SD. *Statistically significant compared to positive control ($p < 0.01$). MIC: Minimum Inhibitory Concentration in mg/mL.

E. faecalis, *E. coli*, *K. pneumoniae* and *C. albicans* [20], on 12 bacterial strains related to gastrointestinal diseases (Hernández *et al*. 2003), as well as inhibitory effect of essential oil on *Mycobacterium tuberculosis* [15].

3.2. Bacterial Kinetics

In bacterial kinetic tests of acetone extract of *T. diffusa*, the Gram-negative strain most susceptible was *E. coli* cc, as a bactericidal effect was observed at concentrations of 3.0 and 2.0 mg/mL, *i.e*., death of 99.9% of microorganisms at 2 and 6 hours of exposure to extract (Figure 1). The same effect was observed in *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853 at 24 hours of exposure to extract at concentration of 3.0 mg/mL. Only a bacteriostatic effect was observed in *S. marcescens* ATCC 14756, as the highest concentrations of extract (3.0 and 4.0 mg/mL) showed a decrease in microbial growth but not its elimination.

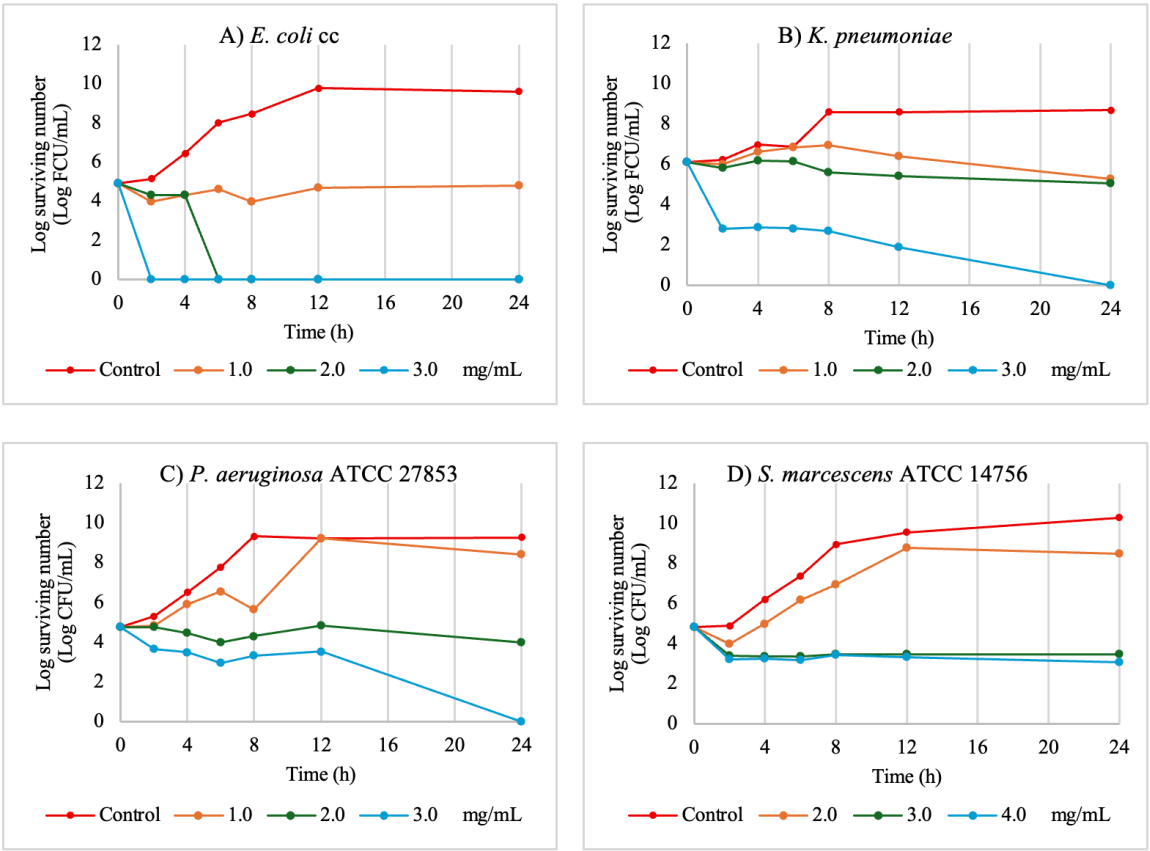

Figure 1. Effect of acetone extract of *T. diffusa* on bacterial death curves in Gram negative strains. The control was tested without extract. Data are represented as the mean ± S.E. (n = 3). In all extract concentrations evaluated, there were statistically significant differences compared to the control.

Of the Gram-positive bacteria, *S. aureus* cc was the most susceptible strain to acetone extract, with a bactericidal effect observed 2 hours after exposure to extract at a concentration of 3.0 mg/mL (Figure 2). The same effect was observed in *E. epidermidis* after 12 hours of exposure. *E. faecalis* was more resistant, with a

bacteriostatic effect observed 24 hours after exposure to 3.0 mg/mL. The difference in time periods in which acetone extract eliminate different microbial populations can be attributed to factors such as microbial morphology and resistance mechanisms of each microorganism, which can vary, even in strains of same species [31]. *E. coli*, from Gram-negative group, and *S. aureus* cc, from Gram-positive group, were strains most susceptible to acetone extract, this effect is significant because these species are cause of a large number of fatal infections worldwide, and they are also among species that have developed a high percentage of resistance to antibiotics such as methicillin, third-generation cephalosporins, ampicillin, clotrimoxazole, and fluoroquinolones [32]. The mechanisms by which this effect occurs remain to be resolved; however, according to chemical composition of acetone extract, which is mainly composed of phenolic compounds such as phenylpropanoids, flavonoids and tannins (**Table 3** and **Table 4**), it is likely that compounds such as those mentioned are responsible for effect. The antibacterial activity of phenolic compounds is due to a combination of mechanisms, such as enzyme inhibition, disruption of cell membrane, induction of oxidative stress, and interference with metabolic processes of microorganisms [33].

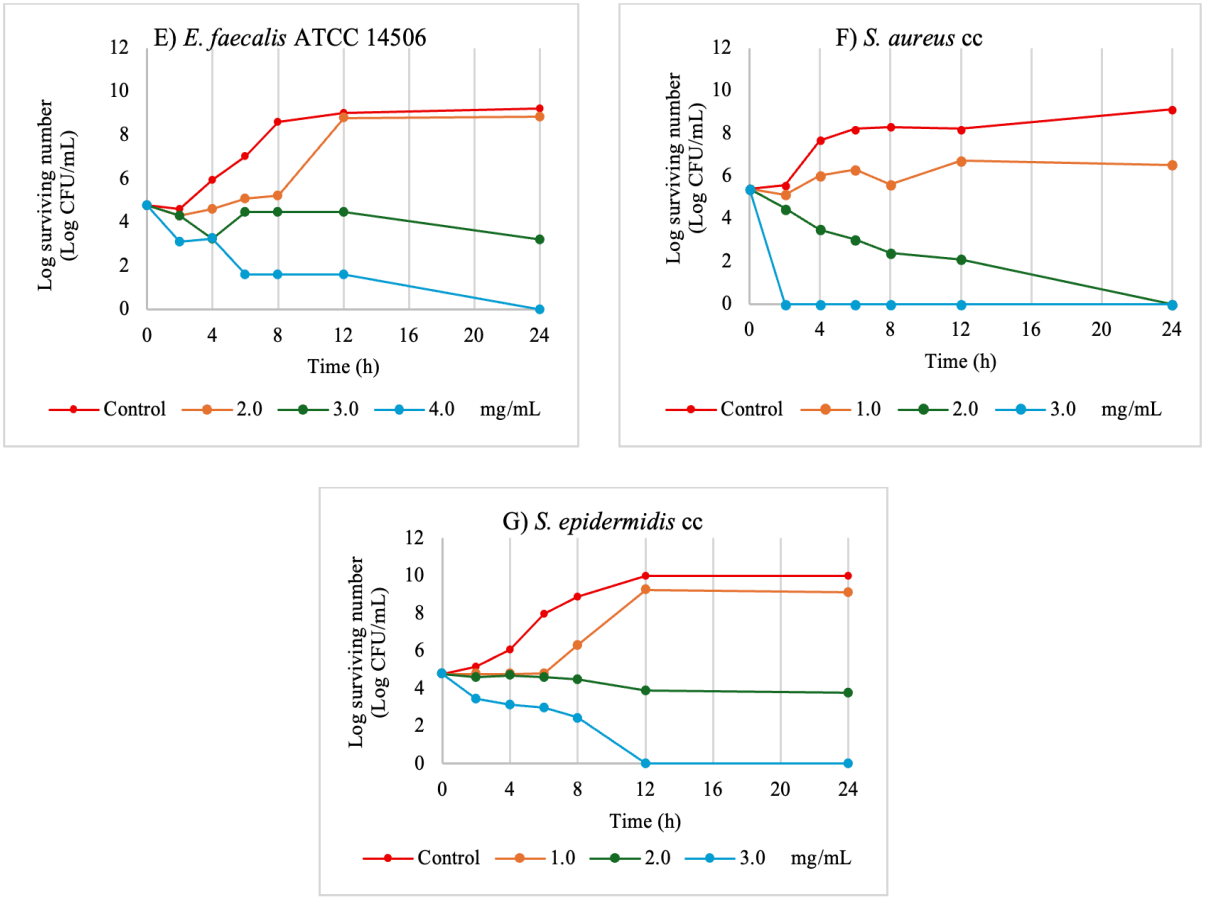

Figure 2. Effect of acetone extract of *T. diffusa* on bacterial death curves in Gram positive strains. The control was tested without extract. Data are represented as the mean ± S.E. (n = 3). In all extract concentrations evaluated, there were statistically significant differences compared to the control.

3.3. Antioxidant Activity

In antioxidant activity tests, all three extracts of *T. diffusa* showed a reducing effect on DPPH and ABTS radicals. The methanolic extract showed a greater effect at lowest concentrations (IC 50 = 29.99 ± 1.84 and 106.02 ± 0.81 μg/mL, respectively) (Table 2), this effect is directly related to the content of phenolic compounds, which was higher in the methanolic extract (60.52 mg GAE/g, Figure 3) compared to the acetone and hexane extracts. This relationship has been mentioned in several studies [34]-[36].

Although the methanolic extract showed a reducing effect on DPPH and ABTS radicals, this was greater for DPPH, presenting a lower IC_{50} value (29.99 ± 1.84 vs 106.02 ± 0.81 μg/mL, respectively), which suggests that there is a difference in the way the compounds contained in extracts react with radicals. In DPPH assay, the radical has a greater affinity for hydrophilic antioxidant components, while $ABTS^{\bullet+}$ radical can interact with both hydrophilic and lipophilic antioxidant compounds [37].

In the ferric reduction assay (FRAP), the methanolic extract also was the most effective, with 569.25 ± 6.78 mgET/g of extract, corresponding to 56.93% iron reduction (Fe^{3+} to Fe^{2+}) (Table 2), suggesting that the phenolic components in extract have the capacity to chelate iron. This has biological importance because, although iron is an important constituent in the function of hemoproteins, such as hemoglobin and myoglobin, cytochromes in electron transport chain, and other proteins, an excess of iron in the body can be toxic to cells and cause oxidative stress [38].

Table 2. Antioxidant activity of *T. diffusa* extracts.

Extract	DPPH	ABTS	FRAP	% of iron reduction
	IC_{50} (μg/mL)	IC_{50} (μg/mL)	mgET/g	
Hexane	729.32 ± 22.93*	1054.60 ± 18.97*	314.42 ± 1.72[a]	31.44 ± 0.17[a]
Acetone	87.11 ± 0.93*	138.76 ± 3.09*	438.77 ± 4.55[b]	43.78 ± 0.45[b]
Methanol	29.99 ± 1.84*	106.02 ± 0.81*	569.25 ± 6.78[c]	56.93 ± 0.67[c]
Quercetin	4.93 ± 0.19	31.00 ± 0.84		

Data expressed as mean ± SD. n = 3. DPPH and ABTS significantly different compared to reference standard (quercetin) (*P < 0.001) by one-way ANOVA. Different letters denote significant differences (P < 0.05). mgET/g: equivalent milligrams of Trolox per gram of extract.

The results of iron-reducing antioxidant power of extracts suggest that polyphenols they contain, mainly in methanolic extract, have the property of binding with iron. This reaction is related to presence of catechol and galloyl groups [39] found in polyphenols such as flavonoids and tannins, which are groups of secondary metabolites identified in methanolic extract of *T. diffusa* in this work (Table 3).

3.4. Phytochemical Screening

In secondary metabolite groups identification tests, flavonoids and steroids were

found in all extracts (Table 3). Acetone and methanol extracts presented similar groups (phenols, flavonoids, tannins and steroids). These secondary metabolites in *T. diffusa* have been reported in other works, where it is mentioned that it contains flavonoids, terpenes [40], tannins and steroids [41].

Table 3. Groups of secondary metabolites present in *T. diffusa* extracts.

Extract	Cum	Phe	Alk	Flv	Tan	Gly	Str	Ter
Hexane	–	–	–	√	–	–	√	√
Acetone	–	√	–	√	√	–	√	–
Methanol	–	√	–	√	√	–	√	–

Cum: coumarins, Phe: phenols, Alk: alkaloids, Flv: flavonoids, Tan: tannins, Gly: glycosides, Str: steroids, Ter: terpenes. √ positive test. – negative test.

3.5. Total Phenol Content

The methanolic extract showed the highest concentration of phenols (Figure 3), with 60.52 mgEAG/g, corresponding to 6.05%, while hexane extract had lowest (0.525%). This result is explained by the fact that polar solvents, such as methanol, dissolve equally polar and ionic solutes [42], including phenolic compounds, whose polarity is due to various OH groups in their structure [43].

In this study, methanolic extract, due to its higher phenol concentration, exhibited highest antioxidant activity, demonstrating the direct relationship between phenol content and antioxidant activity, as previously mentioned in several studies [44]. This occurs because phenols can reduce and stabilize free radicals, which are harmful to cells. Their antioxidant mechanism of action consists of the transfer of hydrogen atoms, electrons, and chelation of transition metals [45].

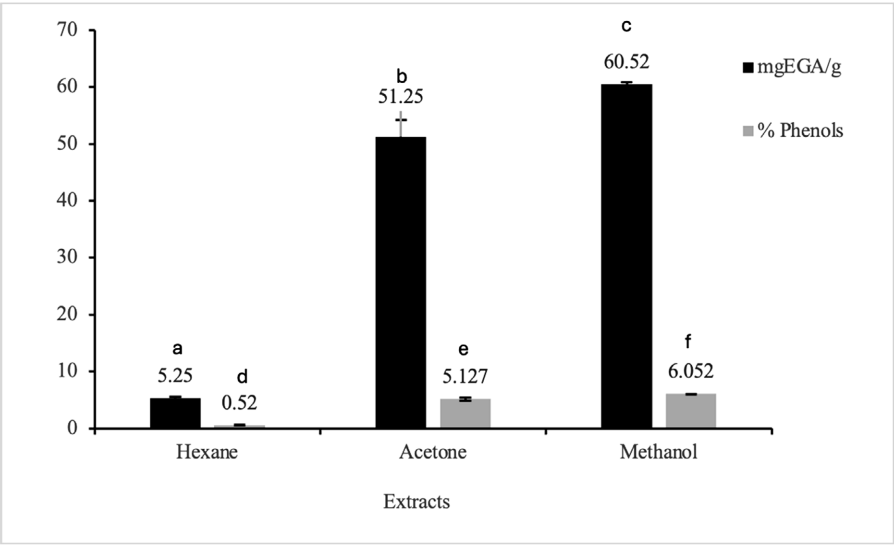

Figure 3. Total phenols of *T. diffusa*. Data expressed as mean ± SD. n = 3. Different letters denote significant differences ($P < 0.05$). mgEGA/g: equivalent milligrams of gallic acid per gram of extract.

3.6. High-Performance Liquid Chromatography

The phenol groups identified in this work were phenylpropanoids and flavonoids, as two large phenolic subgroups. In HPLC analyzes carried out on extracts, it was obtained that phenylpropanoids were most abundant and diverse, 9 were detected in acetone extract and 6 in methanolic extract (Table 4). While flavonoids, although detected, were found in lower quantities (1 in acetone extract and 3 in methanolic extract). These analyzes were carried out by comparing the ultraviolet absorption patterns of mentioned phenol groups. In both extracts, the abundance of phenylpropanoids was more than 90% (1505.4 mUA = 99.56% phenylpropanoids for acetone extract; 953.2 mUA = 94.18% for methanolic extract).

Table 4. Reverse-phase HPLC of acetone and methanol extracts of *T. diffusa* (DAD, λ = 254 nm).

Phenolic compound	RT (min)	UV λ_{max} (nm)	Abundance (mAU)
	Acetone extract		
1	1.988	245 ^{php}	30.2
2	2.435	276 ^{php}	543.0
3	3.028	272 ^{php}	232.0
4	3.175	270 ^{php}	209.0
5	3.555	270, 346^{php}	191.0
6	3.701	270,348^{php}	166.0
7	4.368	270, 346^{php}	85.9
8	6.021	270, 346^{php}	33.1
9	7.615	284, 346^{php}	15.2
10	10.281	238, 278, 360^{flv}	6.6
	Methanolic extract		
1	2.446	280^{php}	338
2	3.073	280^{php}	216
3	3.326	280^{php}	163
4	3.766	278^{php}	129
5	4.486	258^{php}	75.8
6	6.393	256, 284^{php}	31.4
7	7.273	258, 328, 364^{flv}	24.5
8	10.619	278, 326, 362^{flv}	21.5
9	15.446	238, 274, 364^{flv}	12.9

RT: retention time, php: phenylpropanoid, flv: flavonoid, λmax: maximum absorbance, mAU: milliabsorbance units.

Phenylpropanoids are formed by an aromatic ring and a propane group [46] and may also have hydroxyl groups. They are part of group of simplest phenolic compounds and are precursors in biosynthesis of flavonoids, stilbenes, and coumarins, which together are among the main groups of secondary plant metabolites with pharmacological effects [47]. Phenylpropanoids and their derivatives have shown a potent effect on bacteria of genera *Escherichia*, *Pseudomonas*, *Staphylo-*

coccus, among others. An antioxidant effect has also been reported, due to their ability to stabilize free radicals, inhibit lipid peroxidation, and chelate metal ions [48], effects that have been corroborated in this work. However, a more specific analysis is still needed to elucidate the precise chemical structure of each of phenylpropanoid and flavonoid constituents in bioactive extracts, corresponding to acetate extract, which was more effective against bacteria, and the methanolic extract, which showed greater antioxidant activity.

4. Conclusion

In this study, the antibacterial and antioxidant effects of *T. diffusa* were evaluated. The acetone extract showed the greatest antimicrobial activity, inhibiting the growth of seven bacterial strains, of which *E. coli* cc and *S. aureus* cc were the most susceptible. In these strains, the extract caused death of 99.9% of bacterial population after two hours of exposure. The methanolic extract showed greatest antioxidant effect on DPPH and ABTS radicals and on ferric reduction reaction (FRAP). This extract also presented highest concentration of phenols, most of which correspond to phenylpropanoid structures. The compounds identified in two bioactive extracts (acetone and methanol) were phenols, flavonoids, tannins, and steroids. The results suggest that *T. diffusa* is a natural product with potential to yield active ingredients for treatment of infectious diseases and those caused by oxidative stress, although its isolation remains pending. Future research will need to evaluate the biological effects of extracts on *in vivo* models and their toxicity to provide a more comprehensive scientific perspective.

Acknowledgements

We acknowledge to the inhabitants of Tehuacan-Cuicatlán Valley, Puebla (Mexico), also to Master of Science Luis Barbo Hernández Portilla, for his valuable collaboration in HPLC tests, and to Mr. Hector Cervantes Maya for his collaboration in field collection.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Liu, Q., Liu, M., Liang, W., Li, X., Jing, W., Chen, Z., *et al.* (2025) Global Distribution and Health Impact of Infectious Disease Outbreaks, 1996-2023: A Worldwide Retrospective Analysis of World Health Organization Emergency Event Reports. *Journal of Global Health*, **15**, Article No. 04151. https://doi.org/10.7189/jogh.15.04151

[2] Salam, M.A., Al-Amin, M.Y., Salam, M.T., Pawar, J.S., Akhter, N., Rabaan, A.A., *et al.* (2023) Antimicrobial Resistance: A Growing Serious Threat for Global Public Health. *Healthcare*, **11**, Article 1946. https://doi.org/10.3390/healthcare11131946

[3] Patangia, D.V., Anthony Ryan, C., Dempsey, E., Paul Ross, R. and Stanton, C. (2022) Impact of Antibiotics on the Human Microbiome and Consequences for Host Health. *MicrobiologyOpen*, **11**, e1260. https://doi.org/10.1002/mbo3.1260

[4] Sharifi-Rad, M., Anil Kumar, N.V., Zucca, P., Varoni, E.M., Dini, L., Panzarini, E., *et al*. (2020) Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Frontiers in Physiology*, **11**, Article ID: 694. https://doi.org/10.3389/fphys.2020.00694

[5] Ghosh, P., Goswami, S., Roy, S., Das, R., Chakraborty, T. and Ray, S. (2025) Comparative *in Vitro* Antioxidant and Antibacterial Activities of Leaf Extract Fractions of Crimson Bottlebrush, Callistemon Citrinus (Curtis.) Skeels. *The Microbe*, **8**, Article 100445. https://doi.org/10.1016/j.microb.2025.100445

[6] Semarnat (2021) Plantas medicinales de méxico. https://goo.su/JZvylo

[7] Mandal, M.K. and Domb, A.J. (2024) Antimicrobial Activities of Natural Bioactive Polyphenols. *Pharmaceutics*, **16**, Article 718. https://doi.org/10.3390/pharmaceutics16060718

[8] Pandey, K.B. and Rizvi, S.I. (2009) Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxidative Medicine and Cellular Longevity*, **2**, 270-278. https://doi.org/10.4161/oxim.2.5.9498

[9] Martínez-Ávila, G.C.G., Aguilar-Zarate, P. and Rojas, R. (2021) Currently Applied Extraction Processes for Secondary Metabolites from Lippia Turbinata and *Turnera diffusa* and Future Perspectives. *Separations*, **8**, Article 158. https://doi.org/10.3390/separations8090158

[10] Urbizu-González, A.L., Castillo-Ruiz, O., Martínez-Ávila, G.C.G. and Torres-Castillo, J.A. (2017) Natural Variability of Essential Oil and Antioxidants in the Medicinal Plant *Turnera diffusa*. *Asian Pacific Journal of Tropical Medicine*, **10**, 121-125. https://doi.org/10.1016/j.apjtm.2017.01.013

[11] Reyes-Becerril, M., Maldonado, M., Vimolmangkang, S. and Angulo, C. (2024) *In Vivo* and *ex Vivo* Studies Support the Immunostimulant and Immunoprotective Effect of Damiana (*Turnera diffusa* Willd) in Almaco Jack (*Seriola rivoliana*). *Fish & Shellfish Immunology*, **146**, Article 109369. https://doi.org/10.1016/j.fsi.2024.109369

[12] Biblioteca Digital de la Medicina Tradicional Mexicana (2009) Damiana. *Turnera diffusa* Willd. Atlas de las plantas de la medicina tradicional Mexicana. Instituto Nacional Indigenista. https://goo.su/ib4CJpW

[13] Szewczyk, K. and Zidorn, C. (2014) Ethnobotany, Phytochemistry, and Bioactivity of the Genus Turnera (Passifloraceae) with a Focus on Damiana—*Turnera diffusa*. *Journal of Ethnopharmacology*, **152**, 424-443. https://doi.org/10.1016/j.jep.2014.01.019

[14] Hernández, T., Canales, M., Avila, J.G., Duran, A., Caballero, J., Vivar, A.R.D., *et al*. (2003) Ethnobotany and Antibacterial Activity of Some Plants Used in Traditional Medicine of Zapotitlán De Las Salinas, Puebla (México). *Journal of Ethnopharmacology*, **88**, 181-188. https://doi.org/10.1016/s0378-8741(03)00213-7

[15] Bueno, J., Escobar, P., Martínez, J.R., Leal, S.M. and Stashenko, E.E. (2011) Composition of Three Essential Oils, and Their Mammalian Cell Toxicity and Antimycobacterial Activity against Drug Resistant-Tuberculosis and Nontuberculous Mycobacteria Strains. *Natural Product Communications*, **6**, 1743-1748. https://doi.org/10.1177/1934578x1100601143

[16] Zhao, J., Dasmahapatra, A.K., Khan, S.I. and Khan, I.A. (2008) Anti-Aromatase Activity of the Constituents from Damiana (*Turnera diffusa*). *Journal of Ethnopharmacology*, **120**, 387-393. https://doi.org/10.1016/j.jep.2008.09.016

[17] Avelino-Flores, M.C., Cruz-López, M.C., Jiménez-Montejo, F.E. and Reyes-Leyva, J. (2015) Cytotoxic Activity of the Methanolic Extract of *Turnera diffusa* Willd on Breast Cancer Cells. *Journal of Medicinal Food*, **18**, 299-305. https://doi.org/10.1089/jmf.2013.0055

[18] Taha, M.M.E., Salga, M.S., Ali, H.M., Abdulla, M.A., Abdelwahab, S.I. and Hadi, A.H.A. (2012) Gastroprotective Activities of *Turnera diffusa* Willd. Ex Schult. Revisited: Role of Arbutin. *Journal of Ethnopharmacology*, **141**, 273-281. https://doi.org/10.1016/j.jep.2012.02.030

[19] Parra-Naranjo, A., Delgado-Montemayor, C., Fraga-López, A., Castañeda-Corral, G., Salazar-Aranda, R., Acevedo-Fernández, J., *et al*. (2017) Acute Hypoglycemic and Antidiabetic Effect of Teuhetenone a Isolated from *Turnera diffusa*. *Molecules*, **22**, Article 599. https://doi.org/10.3390/molecules22040599

[20] Báez-Parra, K.M., Soto-Beltrán, M., López-Cuevas, O., Heredia, J.B., Alcaraz-Meléndez, L. and Angulo-Escalante, M.A. (2019) *In Vitro* Antimicrobial Activity of Methanolic and Hexanic Extracts of *Turnera diffusa* against Common Urinary Pathogens. *Revista Bio Ciencias*, **6**, e670.

[21] Delgado-Montemayor, C., Cordero-Pérez, P., Torres-González, L., Salazar-Cavazos, M.d.l.L., Saucedo, A.L., Paniagua-Vega, D., *et al*. (2022) Development of a Hepatoprotective Herbal Drug from *Turnera diffusa*. *Evidence-Based Complementary and Alternative Medicine*, **2022**, Article ID: 5114948. https://doi.org/10.1155/2022/5114948

[22] Hudzicki, J. (2009) Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. American Society for Microbiology, 1-23. https://goo.su/r9dk

[23] Wiegand, I., Hilpert, K. and Hancock, R.E.W. (2008) Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nature Protocols*, **3**, 163-175. https://doi.org/10.1038/nprot.2007.521

[24] Candelaria-Dueñas, S., Serrano-Parrales, R., Ávila-Romero, M., Meraz-Martínez, S., Orozco-Martínez, J., Ávila-Acevedo, J.G., *et al*. (2021) Evaluation of the Antimicrobial Activity of Some Components of the Essential Oils of Plants Used in the Traditional Medicine of the Tehuacán-Cuicatlán Valley, Puebla, México. *Antibiotics*, **10**, Article 295. https://doi.org/10.3390/antibiotics10030295

[25] Baliyan, S., Mukherjee, R., Priyadarshini, A., Vibhuti, A., Gupta, A., Pandey, R.P., *et al*. (2022) Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus Religiosa. *Molecules*, **27**, Article 1326. https://doi.org/10.3390/molecules27041326

[26] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999) Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology and Medicine*, **26**, 1231-1237. https://doi.org/10.1016/s0891-5849(98)00315-3

[27] Benzie, I.F.F. and Strain, J.J. (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, **239**, 70-76. https://doi.org/10.1006/abio.1996.0292

[28] Robles-García, M.A., Aguilar, A.J., Gutiérrez-Lomelí, M., Rodríguez-Félix, F., Morales-Del-Río, J.A., Guerrero-Medina, P.J., *et al*. (2016) Identificación cualitativa de metabolitos secundarios y determinación de la citotoxicidad de extractos de Tempisque (*Sideroxylum capiri* Pittier). *Revista de Ciencias Biológicas y de la Salud*, **18**, 3-8. https://doi.org/10.18633/biotecnia.v18i3.328

[29] Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999) Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. *Methods in Enzymology*, **299**, 152-178. https://doi.org/10.1016/s0076-6879(99)99017-1

[30] Vaou, N., Stavropoulou, E., Voidarou, C., Tsakris, Z., Rozos, G., Tsigalou, C., *et al*. (2022) Interactions between Medical Plant-Derived Bioactive Compounds: Focus on

Antimicrobial Combination Effects. *Antibiotics*, **11**, Article 1014. https://doi.org/10.3390/antibiotics11081014

[31] Reygaert, W.C. (2018) An Overview of the Antimicrobial Resistance Mechanisms of Bacteria. *AIMS Microbiology*, **4**, 482-501. https://doi.org/10.3934/microbiol.2018.3.482

[32] WHO (2022) Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report 2022. WHO. https://goo.su/7dpQSK

[33] Dembińska, K., Shinde, A.H., Pejchalová, M., Richert, A. and Swiontek Brzezinska, M. (2025) The Application of Natural Phenolic Substances as Antimicrobial Agents in Agriculture and Food Industry. *Foods*, **14**, Article 1893. https://doi.org/10.3390/foods14111893

[34] Jacobo-Velázquez, D.A. and Cisneros-Zevallos, L. (2009) Correlations of Antioxidant Activity against Phenolic Content Revisited: A New Approach in Data Analysis for Food and Medicinal Plants. *Journal of Food Science*, **74**, R107-R113. https://doi.org/10.1111/j.1750-3841.2009.01352.x

[35] Piluzza, G. and Bullitta, S. (2011) Correlations between Phenolic Content and Antioxidant Properties in Twenty-Four Plant Species of Traditional Ethnoveterinary Use in the Mediterranean Area. *Pharmaceutical Biology*, **49**, 240-247. https://doi.org/10.3109/13880209.2010.501083

[36] Sulaiman, S.F., Yusoff, N.A.M., Eldeen, I.M., Seow, E.M., Sajak, A.A.B., Ooi, K.L., *et al*. (2011) Correlation between Total Phenolic and Mineral Contents with Antioxidant Activity of Eight Malaysian Bananas (Musa Sp.). *Journal of Food Composition and Analysis*, **24**, 1-10. https://doi.org/10.1016/j.jfca.2010.04.005

[37] Bibi Sadeer, N., Montesano, D., Albrizio, S., Zengin, G. and Mahomoodally, M.F. (2020) The Versatility of Antioxidant Assays in Food Science and Safety—Chemistry, Applications, Strengths, and Limitations. *Antioxidants*, **9**, Article 709. https://doi.org/10.3390/antiox9080709

[38] Scarano, A., Laddomada, B., Blando, F., De Santis, S., Verna, G., Chieppa, M., *et al*. (2023) The Chelating Ability of Plant Polyphenols Can Affect Iron Homeostasis and Gut Microbiota. *Antioxidants*, **12**, Article 630. https://doi.org/10.3390/antiox12030630

[39] Khokhar, S. and Owusu Apenten, R.K. (2003) Iron Binding Characteristics of Phenolic Compounds: Some Tentative Structure-Activity Relations. *Food Chemistry*, **81**, 133-140. https://doi.org/10.1016/s0308-8146(02)00394-1

[40] Zhao, J., Pawar, R.S., Ali, Z. and Khan, I.A. (2007) Phytochemical Investigation of *Turnera diffusa*. *Journal of Natural Products*, **70**, 289-292. https://doi.org/10.1021/np060253r

[41] Oliveira-Silva, M., Santos, M.H., Nunes, X., Fernandes-Santos, E. and Bispo, E. (2020) Phytochemical Analysis of *Turnera diffusa* Willd. *International Journal for Innovation Education and Research*, **8**, 402-411. https://doi.org/10.31686/ijier.vol8.iss11.2804

[42] Su, G., Yang, L., Liu, S., Song, J., Jiang, W. and Jin, X. (2024) Review on Factors Affecting Nanofluids Surface Tension and Mechanism Analysis. *Journal of Molecular Liquids*, **407**, 125159. https://doi.org/10.1016/j.molliq.2024.125159

[43] Taheri, A. and Jafari, S.M. (2019) Gum-Based Nanocarriers for the Protection and Delivery of Food Bioactive Compounds. *Advances in Colloid and Interface Science*, **269**, 277-295. https://doi.org/10.1016/j.cis.2019.04.009

[44] Džarić, T., Petrović, D. and Božović, M. (2025) Antioxidant Activity and Total Phenolic Content of Different Extracts from *Rosa canina* L. Fruits. *Natural Product Commu-*

nications, **20**, 1-11. https://doi.org/10.1177/1934578x251369590

[45] Zeb, A. (2020) Concept, Mechanism, and Applications of Phenolic Antioxidants in Foods. *Journal of Food Biochemistry*, **44**, e13394. https://doi.org/10.1111/jfbc.13394

[46] Yingngam, B. (2023) Modern Solvent-Free Microwave Extraction with Essential Oil Optimization and Structure-Activity Relationships. *Studies in Natural Products Chemistry*, **77**, 365-420.

[47] Lin, Y. and Yan, Y. (2014) Biotechnological Production of Plant-Specific Hydroxylated Phenylpropanoids. *Biotechnology and Bioengineering*, **111**, 1895-1899. https://doi.org/10.1002/bit.25237

[48] Neelam, Khatkar, A. and Sharma, K.K. (2019) Phenylpropanoids and Its Derivatives: Biological Activities and Its Role in Food, Pharmaceutical and Cosmetic Industries. *Critical Reviews in Food Science and Nutrition*, **60**, 2655-2675. https://doi.org/10.1080/10408398.2019.1653822