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ARTICLE

In vitro Antibacterial Activity of *Moringa oleifera* Ethanolic Extract against Tomato Phytopathogenic Bacteria

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ABSTRACT

The tomato (*Solanum lycopersicum* L.) is one of the world's most important vegetable crops. Still, phytopathogenic bacteria affect the yield and quality of tomato cultivation, like *Agrobacterium tumefeciens* (*At*), *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), *Pseudomonas syringae pv. tomato* (*Pst*), *Ralstonia solanacearum* (*Rs*), and *Xanthomonas axonopodis* (*Xa*). Synthetic chemical products are used mostly on disease plant control, but overuse generates resistance to bacterial control. This study aimed to evaluate the *in vitro* antibacterial activity of the ethanolic extract of *Moringa oleifera* Lam. leaves against *At*, *Cmm*, *Pst*, *Rs*, and *Xa*, as well as information about this plant species' chemical composition. Antibacterial activity against pathogens observed by microplate technique, phytochemical screening, and FTIR analysis revealed different bio-active compounds on ethanolic extracts with antibacterial activity. The growth inhibition rate ranged between 0.08% and 99.94%. The inhibitory concentration, IC_{50}, required to inhibit 50% of *At*, *Cmm*, *Pst*, *Rs*, and *Xa* bacterial growth, was 276.67, 350.48, 277.85, 351.49, and 283.22 mg/L, respectively. Inhibition of phytopathogen bacteria's growth increased as the concentrations of the extract also increased. *Moringa oleifera* extract can be recommended as a potent bio-bactericide.

KEYWORDS

Biological control; secondary metabolites; tomato crop; inhibitory concentration; botanical extract

1 Introduction

The tomato (*Solanum lycopersicum* Linnaeus; Solanaceae) is the second most important vegetable crop worldwide in economic terms, after the potato [1]. Also, it is the most highly consumed vegetable due to its status as the main ingredient in a large variety of raw, cooked, or processed foods [2]. There are 5.802 million tomato hectares globally, with an average yield of 55.55 t/ha [3]. There are abiotic and biotic factors that can affect the yield and quality of the tomato crop, such as the attack of pests and diseases [2]. The phytopathogenic bacteria like *Agrobacterium tumefeciens* (*At*), *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*), *Pseudomonas syringae pv. tomato* (*Pst*), *Ralstonia solanacearum* (*Rs*), and

Xanthomonas axonopodis (*Xa*), cause severe damage and economic losses on tomato crops [4]. They are considered as causal agents of bacterial blight, freckle, canker, root, and crown gall, respectively [5,6].

The control of phytopathogenic bacteria depends mainly on synthetic chemical products like streptomycin and copper, but some bacteria develop resistance, limiting chemical control's efficiency and effectiveness [7]. Some investigations have reported disease control by ecological methods based on disturbing host-pathogen relations [8]. Several studies documented that the botanical extracts appear to be a better alternative, as they are known to have a minimal impact in both the environment and the consumer than synthetic pesticides [9].

Moringa oleifera Lam. (Moringaceae) is native to India and Africa. In some parts of the world, it is known as the tree of life, horseradish tree, or drumstick tree. *Moringa oleifera* is widely used in industry for its nutritional and medicinal value. Aqueous and ethanolic *M. oleifera* extracts from seeds, stem bark, leaves, or root bark, reported an antimicrobial potential, with inhibitory effects on Gram-positive species (*Staphylococcus aureus* and *Enterococcus faecalis*) over Gram-negative species (*Escherichia coli*, *Salmonella*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Aeromonas caviae*) for medical applications [10,11]. Additionally, Goss et al. [12] documented that the leaf, bark, and seed extracts of *M. oleifera* reduced leaf defoliation caused by *Xanthomonas. campestris pv. campestris* in rape (*Brassica napus* L.; Brassicaceae) under field conditions in agricultural areas.

The bioactivity compounds of *M. oleifera* have been widely studied in medicine and the food industry by promising antimicrobial properties [11]. In contrast, it has been investigated as a growth stimulator of plant or fungal control in agronomic areas. However, there are few studies on analyzing the control of phytopathogenic bacteria [12]. This study aimed at evaluating the *in vitro* antibacterial activity of the ethanolic extract of *M. oleifera* leaves against *A. tumefeciens*, *C. michiganensis* subsp. *michiganensis*, *P. syringae pv. tomato*, *R. solanacearum*, and *X. axonopodis*, as well as to obtain information about the extract's chemical composition.

2 Materials and Methods

2.1 Plant Material

Moringa oleifera leaves were provided by the Institute of Applied Ecology (IAE) at the Universidad Autónoma de Tamaulipas (UAT), in May 2018. Taxonomic identification of genus and species was confirmed by the IAE-UAT. Samples were labeled and transported in brown paper bags inside iceboxes to the Parasitology Department at the Universidad Autonoma Agraria Antonio Narro (UAAAN). Immediately, the vegetal tissue was dehydrated using a conventional oven (Quincy lab, USA model 20GCE-LT) at 60°C for three days until constant weight. Thereafter, samples were ground (miller CUISINART, USA, model DBM-8) to obtain 1 mm particle sizes. The powder was stored in dark bottles at room temperature until the extraction was performed [13].

2.2 Plant Extract

Fourteen gram samples of homogenized dried powder of leaves of *M. oleifera* were mixed with 200 mL of absolute ethanol at room temperature for three days with a magnetic stirrer in darkness. The mixture leaked with Whatman No. 1 filter paper. The extract evaporated until the solvent was removed by rotary evaporation (IKA-RV 10 digital V, USA Inc., USA), under reduced pressure at temperatures below 40°C. Finally, the remaining ethanol was eliminated by placing the flask on the drying oven until constant weight [14]. The extract was preserved in Eppendorf tubes and placed in a freezer at –10°C until use in the bioassays.

2.3 Phytochemical Analysis of Extracts

Qualitative screening was carried out to obtain the chemical constituents of extracts by standard methods. They were evaluated in the study by several tests for: Alkaloids (Dragendorff's and

Sonheschain's reagent), carbohydrates (Molisch's reagent), carotenoids (H_2SO_4 and $FeCl_3$ reagents), coumarins (Erlich's reagent), flavonoids (Shinoda's reagent and NaOH at 1%), free reducing sugars (Fehling's and Benedict's reagent), cyanogenic glycosides (Grignard's reagent), purines (HCl test), quinones (NH_4OH and H_2SO_4 reagents by anthraquinones, and Börntraguer's test by benzoquinone), saponins (Frothing test, Bouchard reagent for steroidal saponins and Rosenthaler reagent), terpenoids (Ac_2O reagent), soluble starch (KOH and H_2SO_4 test) and tannins ($FeCl_3$ and Ferrocyanide reagents) [15–17].

2.4 Determination of Total Phenols Content (TPC)

Ethanolic extract of *M. oleifera* was determined by the Folin-Ciocalteu method. Briefly, 20 µL of samples were mixed with 120 µL of Na_2CO_3 (15% w/v), 30 µL of Folin-Ciocalteu reagent, and 400 µL of water. The reaction performed at 37°C for 45 min. Thereafter, the absorbance was read at 760 nm in a microplate reader (Thermo Scientific™ Multiskan™ GO, USA). The standard used was Gallic acid (FagaLab) at concentrations of 100 to 2000 mg/mL to plot a standard curve to be used in calibration. TPC was expressed as milligram Gallic acid equivalent (mg GAE)/g dry weight plant material [14].

2.5 Determination of Total Flavonoids Content (TFC)

The assay was performed by the aluminum chloride method. The ethanolic extract was mixed with 0.1 mL of 10% (w/v) aluminum chloride hexahydrate, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. After 40 min incubation period at room temperature, the reaction mixture's absorbance was determined spectrophotometrically at 410 nm (Thermo Scientific™ Multiskan™ GO, USA). The standard used was quercetin (Sigma Aldrich) at concentrations of 100 to 2000 mg/mL to plot a standard curve to be used in calibration. TFC expressed as milligram quercetin (mg QE)/g dry weight plant material [18].

2.6 Antioxidant Capacity of Extracts (ACE)

Theethanolic extract was carried out by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay, according to Kumar et al. [18]. DPPH 60 µM reagent solution was prepared, and 2950 µL of that solution was added to 50 µL of sample extract. The mixture was shaken and incubated for 30 min in the dark at room temperature by continuous monitoring of the absorption decrease at 517 nm. The control solution contained 100 µL of distilled water. The ACE activity was expressed as an inhibition percentage by the following equation:

$$\text{Percentage of inhibition} = (1 - (As / Ac)) \times 100 \quad (1)$$

where *Ac* and *As* is the absorbance of the control solution and the absorbance of the sample solution, respectively.

2.7 Isolation of Bacterial Strains

Bacteria's (*At, Cmm, Pst, Rs*, and *Xa*) isolated from tomato plants presented disease symptoms as bacterial blight, freckle, canker, root, and crown gall, respectively. Samples were identified and labeled to be transported at the Parasitology Department at the UAAAN. Samples were disinfected for 20 s with ethanol at 70% concentration, then during 10 min in sodium hypochlorite solution at 2%. Finally, all samples were washed with sterile water. Samples were dissected aseptically into small segments and macerated in 10 ml NaCl solution at 0.85%. Tissue extracts were serially diluted and plated in triplicate onto King's B-Agar to isolate bacteria on Petri dishes at 30°C for 24–72 h [19]; isolated colonies were stored in a sterile glycerol solution at 20% at –20°C.

2.8 Bacterial's Strain Confirmation by the Polymerase Chain Reaction (PCR)

Strains were grown for three days on Nutrient Dextrose Agar (NDA). Genomic DNA was extracted by the Frederick et al. [20] method. Primers and reactions were specific to *Cmm:* Specific primers were Cmm5 and Cmm6 at 55°C alignment temperature [21]; for *Rs* PEHA3 and PEHA6 were used at 70°C [22].

For *Xa*, primers were BSX1 and BSX2 at 63°C [23]; for *Pst*, we employed primers B1 and B2 at 60°C [24]. Finally, the oligonucleotide sequences for *At* were VirD2A and VirD2E at 50°C (Tolba & Soliman 2014). All reactions were carried out to a final volume of 25 µL (2.5 µL 10 × PCR buffer, 3 mM $MgCl_2$, 0.5 mM dATP, dCTP, dTTP, dGTP, 0.4 µM primers, 0.8 µM probe, 1 U Taq polymerase and 1 µL DNA template). The reactions took place for 40 cycles at 95°C for 10 min, 94°C for 15 s, and respective temperature alignment for 1 min [25]. The amplified products were separated on 1.5% agarose gels including ethidium bromide (0.5 $\mu g.mL^{-1}$) for 2 h at a 6 V cm^{-1} constant voltage in Tris/Borate/EDTA (TBE) buffer [21].

2.9 Preparation of the Bacterial Suspension

For *in-vitro* assays, bacterial suspensions of *At, Cmm, Pst, Rs*, and *Xa* were prepared in Nutrient Broth Medium (NBM) on a shaker incubator at 26°C ± 1°C for 24 h. Suspensions were adjusted to 1×10^8 colony forming units (CFU) mL^{-1}, according to McFarland standards, which corresponds to a wavelength of 600 nm equal to 0.283 (A 600 nm = 0.283) [26].

2.10 Bacteria Inhibition Microplate Assay

Round-shaped well bottom microplates (96-wells) were used. All bottoms were supplemented by 100 µL of NBM, with 2, 3, 5-triphenyl tetrazolium chloride (TTC, tetrazolium red, Sigma T-8877, St. Louis, USA.) as an indicator at 0.01% (w/v), except on the first column. Later, 100 µL of resuspended *M. oleifera* extracts at 2000 mg/L were added at the four-column and homogenized; then, serial dilutions at 50% were conducted to obtain concentrations starting at 1000 until 3.9 mg/L. Thereafter, 100 µL of bacterial suspension (1×10^8 CFU/mL) were put in all bottom wells except on the first column. The third column contained NBM, TTC, ethanol, and bacteria without the *M. oleifera* extract as the evaluation's control. Immediately, microplates were covered with their lid and incubated at 28°C overnight. The absorbance of the bacteria inhibition microplate assay was analyzed at 540 nm in the microplate reader (Thermo Scientific™ Multiskan™ GO, USA) controlled with a Thermo Scientific SkanIt software. The assay was carried out in triplicate for each bacteria. The following equation calculated the percent of bacteria inhibition (%):

$$\%\text{Inhibition} = ((A\ control - A\ sample) / A\ control) \times 100 \quad (2)$$

where *A control* is the absorbance of column three, and *A sample* is the absorbance of samples from column four to twelve. The IC_{50} determined the concentration of sample extracts required to inhibit 50% of bacteria growth.

2.11 Infrared Analysis

The ethanol extract of the *M. oleifera* spectra was recorded into a Spotlight 200i Spectrum Two System spectrometer equipment interfaced with a computer equipped with Spectrum 10 ES™. The powder was analyzed by non-destructive diffuse reflection with the help of a micro-cap with a quartz window. The powder was placed directly on the integrating sphere window. The spectra recorded was between 600 and 4000 cm^{-1} with a 4 cm^{-1} nominal resolution by 100 scans, and using an internal gold reference, every scanning was repeated three times.

2.12 Experimental Design

A completely randomized design was used with six treatments [five phytopathogenic bacteria and one control (bacteria without the ethanol extract of *M. oleifera* leaves, view Section 2.10)]. Four concentrations (1000, 500, 250, 125 mg/L) of the extract were applied to each phytopathogenic bacteria, and these were replicated three times (n = 12). In a microplate, all treatments were established. From the fourth column of the microplate, four wells were used to apply the different concentrations per bacteria. One microplate

represented one replicate. In one row of each microplate, one well was used for each concentration per treatment. Eq. (2) was used to calculate the inhibition percentage. Inhibition was recorded at 24 h.

2.13 Statistical Analysis

The assay for determination of the content of total flavonoids (TFC), total phenols (TPC), and antioxidant capacity of extracts (ACE) was conducted in triplicates. The values are expressed as the mean ± standard error (SE). Before analyzing the data, they were converted to percentages according to Eq. (2). Adjustment of residuals to the normal distribution was checked according to the Kolmogorov-Smirnov (K-S) test, and variance homogeneity was checked with the Levene (F) test. The assumptions were not met, and the $Arcsin\sqrt{(x/100)}$ transformation was performed, where x is the inhibition percentage. The transformed data were analyzed using one-way analysis of variance (ANOVA). When F tests were significant, means were compared using the Tukey's HSD test ($P \leq 0.05$) [27]. Probit analysis was used to estimate the inhibitory concentration [$IC_{50(90)}$], including the CI_{95} values [27,28].

3 Results

3.1 Bacterial's Strain Confirmation

Primers and probe were specific to each studied pathogenic bacteria. For *A. tumefeciens* the amplicon size was of 338 base pairs (bp), for *C. michiganensis* ssp. *michiganensis* the amplicon was at 614 bp, for *P. syringae pv. tomato*, the amplicon was obtained at 752 bp; for *R. solanacearum*, the amplicon was at 504 bp, and finally, for *X. axonopodis*, the amplicon was at 425 bp.

3.2 Antibacterial Activity

The Kolmogorov-Smirnov and Levene tests indicated that the residuals were distributed normally, and variances were homogeneous for the inhibition percentage ($P > 0.05$), respectively. Results showed that the ethanol extract of *M. oleifera* leaves inhibited the growth of the phytopathogenic bacteria *At*, *Cmm*, *Pst*, *Rs*, and *Xa*. A significant increase in the inhibition percentage of each studied bacteria was observed as the extract concentration increased: *A. tumefeciens* ($F = 4746.49$; $df = 3, 8$; $P < 0.0001$), *C. michiganensis ssp. michiganensis* ($F = 1650.33$; $df = 3, 8$; $P < 0.0001$), *P. syringae pv. tomato* ($F = 4906.50$; $df = 3, 8$; $P < 0.0001$), *R. solanacearum* ($F = 4405.08$; $df = 3, 8$; $P < 0.0001$), and *X. axonopodis* ($F = 1527.10$; $df = 3, 8$; $P < 0.0001$). Significant differences in the inhibition percentage were observed among the phytopathogenic bacteria at every extract concentration (500 mg/L: $F = 76856.0$; $df = 4, 10$; $P < 0.0001$; 250 mg/L: $F = 95053.7$; $df = 4, 10$; $P < 0.0001$; 125 mg/L: $F = 3.98$; $df = 4, 10$; $P = 0.0348$) (Tab. 1). The only exception was at the concentration of 1 000 mg/L where there were no significant differences ($F = 0.02$; $df = 4, 10$; $P = 0.9992$) among bacteria. The extract was efficient over the five phytopathogenic bacteria (*At*, *Cmm*, *Pst*, *Rs*, and *Xv*) since 1000 mg/L was required to inhibit almost 100% of the growth of the bacteria; by concentration, the extract was more efficient over *Cmm* (Tab. 1).

The inhibitory concentration $IC_{50(90)}$ required to inhibit 50 (90%) of bacterial growth was lower for *At*, followed by *Pst*, *Xa*, *Cmm*, and *Rs*. This is, the ethanol extract of *M. oleifera* leaves required 27.04% less concentration to reduce 50% of the growth of *At* (IC_{50} = 276.67 mg/L) compared to *Rs* (IC_{50} = 351.49 mg/L). This indicates that *At* was the most susceptible and *Rs* the more resistant phytopathogenic bacteria to the extract of *M. oleifera* leaves (Tab. 2).

3.3 Phytochemical Analysis

The total phenols (TPC) and total flavonoids (TFC) concentrations, and the antioxidant activity (ACE) of the extract are shown in Tab. 3.

Table 1: Inhibition percentage* (IP) at different concentrations of the ethanol extract *of Moringa oleifera* leaves against tomato phytopathogenic bacteria

Phytopathogenic bacteria	IP. At 1000 mg/L**	IP. at 500 mg/L	IP. at 250 mg/L	IP. at 125 mg/L	Kolmogorov-Smirnov test	Levene test
Agrobacterium tumefeciens	99.94 ± 0.05aA (89.23 ± 0.76)	73.50 ± 0.01bB (59.01 ± 0.00)	33.94 ± 0.02cC (35.63 ± 0.01)	0.10 ± 0.06bD (1.45 ± 0.75)	Orig: D = 0.46017; P = 0.007614 Transf: D = 0.32536; P = 0.1246	Orig: F = 0.7723; df = 3, 8; P = 0.5413 Transf: F = 1.0005; df = 3, 8; P = 0.4409
Clavibacter michiganensis ssp. *Michiganensis*	99.90 ± 0.10aA (88.95 ± 1.04)	67.34 ± 0.01cB (55.14 ± 0.00)	19.49 ± 0.01dC (26.19 ± 0.00)	0.41 ± 0.020abD (3.00 ± 1.50)	Orig: D = 0.41683, P = 0.02107 Transf: D = 0.32668; P = 0.1218	Orig: F = 0.69; d f = 3, 8; P = 0.5832 Transf: F = 0.6932; df = 3, 8; P = 0.5815
Pseudomonas syringae pv. Tomato	99.91 ± 0.09aA (89.00 ± 0.99)	75.64 ± 0.01aB (60.42 ± 0.01)	35.24 ± 0.02bC (36.41 ± 0.01)	1.25 ± 0.05aD (6.41 ± 0.13)	Orig: D = 0.46414, P = 0.01137 Transf: D = 0.32668; P = 0.1218	Orig: F = 0.565; df = 3, 8; P = 0.6532 Transf: F = 0.8987; df = 3, 8; P = 0.4829
Ralstonia solanacearum	99.92 ± 0.08aA (89.08 ± 0.91)	55.13 ± 0.06eB (47.94 ± 0.03)	18.61 ± 0.05eC (25.55 ± 0.31)	0.08 ± 0.04bD (1.30 ± 0.64)	Orig: D = 0.4562; P = 0.008399 Transf: D = 0.30808; P = 0.1651	Orig: F = 0.1305; df = 3, 8; P = 0.9392 Transf: F = 0.6477; df = 3, 8; P = 0.6061
Xanthomonas axonopodis	99.89 ± 0.11aA (88.88 ± 1.11)	63.13 ± 0.02dB (52.61 ± 0.01)	40.49 ± 0.05aC (39.52 ± 0.02)	0.38 ± 0.2abD (2.87 ± 1.43)	Orig: D = 0.42335, P = 0.01822 Transf: D = 0.31339; P = 0.1517	Orig: F = 0.4495; df = 3, 8; P = 0.7246 Transf: Transf: F = 0.6653; df = 3, 8; P = 0.5964

Note: *Figures in parentheses are arc-sine transformed values; **Means (±SE) within columns and rows followed by different lowercase and uppercase letters, respectively, are significantly different ($P \leq 0.05$; ANOVA and Tukey's HSD). Orig: Original data. Transf: Transformed data

Table 2: Inhibitory concentrations of the ethanol extract of *Moringa oleifera* leaves against tomato phytopathogenic bacteria

Phytopathogenic bacteria	IC_{50} (CI_{95})	IC_{90} (CI_{95})	β	±SE	χ2	Pr > χ2
Agrobacterium tumefeciens	276.67 (247.73–308.75)	533.76 (462.70–644.65)	7.699	0.727	112.12	<0.0001
Clavibacter michiganensis ssp. *Michiganensis*	350.48 (313.55–389.10)	668.67 (586.29–794.56)	7.83	0.75	107.60	<0.0001
Pseudomonas syringae pv. Tomato	277.85 (246.61–311.36)	568.05 (491.03–687.58)	7.07	0.66	112.28	<0.0001
Ralstonia solanacearum	351.49 (312.50–393.92)	719.05 (620.87–870.83)	7.069	0.651	117.75	<0.0001
Xanthomonas axonopodis	283.22 (248.37–321.97)	628.61 (532.44–781.31)	6.34	0.58	116.25	<0.0001

Note: $IC_{50(90)}$–inhibitory concentration in mg/L, causing 50 (90%) inhibition of bacterial growth. CI_{95}%–confidence interval

The bio-active analysis of *M. oleifera* is shown in Tab. 4. The ethanolic extracts contained some phytochemicals such as alkaloids, soluble starch, sugar reducers, carbohydrates, carotenoids, coumarins, flavonoids, cyanogenic glycosides, quinones, saponins, and tannins.

Table 3: Total phenols concentration (TPC), total flavonoids concentration (TFC), and antioxidant capacity of extracts (ACE) present in the ethanolic extract of *Moringa oleifera* leaves

Plant extract 2000 mg/L	TPC. (G mg GAE/100 g dry weight)	TFC. (QE mg/g dry weight)	ACE. Inhibition percentage (%)
Moringa oleifera	1356.57 ± 0.15	1347.77 ± 0.13	98.16 ± 0.09

Table 4: Phytochemical screening analysis of *Moringa oleifera*

Bioactive	Test
Alkaloids	Dragendorff's (+) Sonheschain's (+)
Soluble starch	(+)
Sugar reducers	Fehling's (+) Benedict's (+)
Carbohydrates	Molisch's (+)
Carotenoids	(+)
Coumarins	Erlich's (+)
Flavonoids	Shinoda (+) for flavanone's NaOH at 1% (+) for flavanone's or xanthone
Cyanogenic glycosides	Grignard's (+)
Purines	(-)
Quinones	NH_4OH (+) for anthraquinone H_2SO_4 (+) for anthraquinone Bröntraguer's (+) for benzoquinone
Saponins	Foam (-) Bouchard (+) for steroidal saponins Rosenthaler (-)
Tannins	Jelly (+) $FeCl_3$ (+) for gallic acid Ferrocyanide (+) for phenols
Terpenoids	Ac_2O (-)

Note: (+) indicates presence; (-) indicates absence.

3.4 Infrared Analysis

FT-IR is a powerful technique for elucidating structural bio-active compounds; it has a unique region known as the fingerprint region where bands' position and intensity are specific for every sample. The significant peaks identifiable in Fig. 1 are described in Tab. 5.

4 Discussion

In this work, the ethanolic extract of *M. oleifera* leaves showed an inhibitory effect over the phytopathogenic bacteria, *At*, *Pst*, *Xa*, *Cmm*, and *Rs*. The inhibition percentage ranged from 0.08% to 100%. Inhibition of the phytopathogenic bacteria growth increased as the concentration of the extract also increased. Al husnan et al. [11] found that the aqueous extract of *M. oleifera* leaves has both antibacterial

and antifungal activity. They assessed both activities by two methods (ELISA reader and inhibition zone). In the antibacterial activity, ELISA reader, at 100 mg/L, reported maximum inhibition rates between 65.3% and 85.9%, and inhibition zone, between 23 mm to 12.5 mm. In the antifungal activity, values for those variables were from 20.3% to 80% and 6.6 mm to 18 mm, respectively. Vinoth et al. [29] documented that the antibacterial activity of the ethanol extract of *M. oleifera* leaves showed a range of inhibition between 13 mm to 8 mm at a concentration of 100 mg/L. The results of our work are more inhibiting than those reported by Al husnan et al. [11] and Vinoth et al. [29]. However, the extraction and evaluation methodologies, although very similar, present differences that may contribute to explain the different results.

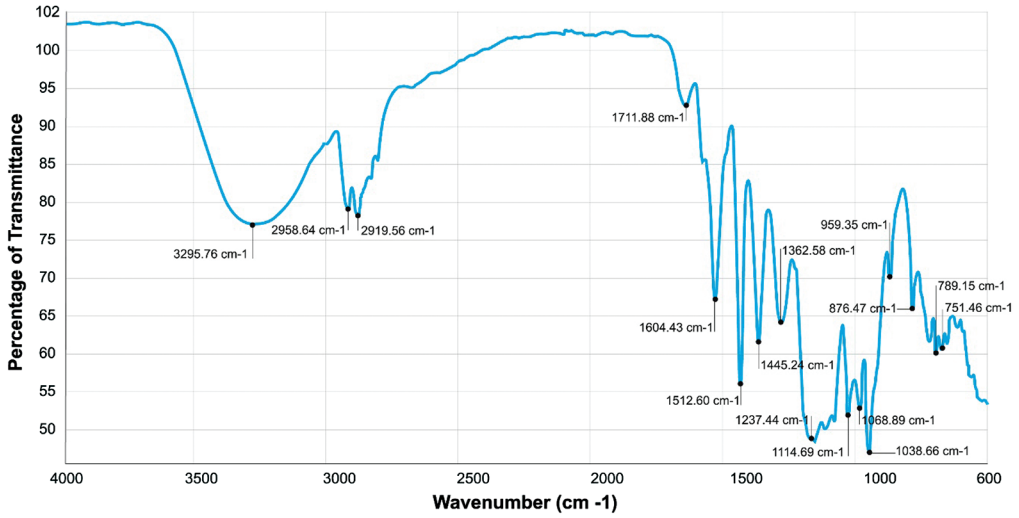

Figure 1: Infrared analysis of *Moringa oleifera* ethanolic extract

Table 5: Stretching and interpretation of the Infrared spectrum analysis of the *Moringa oleifera* ethanolic extract

Peak number	X (cm^{-1})	Y (%T)	Stretching and interpretation
1	3295.76	75.63	O-H stretching alcohol group
2	2958.64	77.42	C-H stretching methyl group
3	2919.56	76.5	N-H stretching alkyl group
4	1711.88	90.07	C=0 stretching aliphatic ketones or esters are identifiable
5	1604.43	66.35	C=0 stretching tertiary amides
6	1512.6	56.03	N=0 stretching Ar-No1
7	1445.24	61.1	C-H def -CH_2-
8	1362.58	63.47	C-H def C-H
9	1237.44	48.75	C-O stretching C=C-O-C, primary alcohol
10	1114.69	51.95	C-O stretching secondary alcohol
11	1068.89	52.83	C-O stretching C=C-O-C
12	1038.66	47.47	C-O stretching C=C-O-C
13	959.35	68.64	C-O stretching C=C-O-C

(Continued)

Table 5 (continued).

Peak number	X (cm^{-1})	Y (%T)	Stretching and interpretation
14	876.47	64.85	N-H stretching amine $–NH_2$ or –NH-
15	812.57	61	N-H stretching amine $–NH_2$ or –NH-
16	789.15	59.71	C-C stretching and C=C bonds in aromatic rings
17	769.64	59.9	C-C stretching and C=C bonds in aromatic rings
18	751.46	60.86	C-C stretching and C=C bonds in aromatic rings

Moringa oleifera extracts can be effectively implemented to suppress *A. tumefeciens, C. michiganensis* ssp. *michiganensis, P. syringae pv. tomato, R. solanacearum*, and *X. axonopodis* pathogens in tomato crop. However, it is necessary to carry out more experiments at the fieldand greenhouse to generate an integrated disease control program.

The leaf extract of *M. oleifera* showed different secondary metabolites, like phenols, flavonoids, and antioxidant activity. Phenolic compounds are part of the secondary metabolites involved in plant defense against pathogens [30]. Shanmugavel et al. [31] reported 627 ± 12.26 mg GAE/100 g for TPC, and 22.16 ± 1.54 mg QE/g for TFC; their results were then lowerthan those found in this research. Nevertheless, Guzmán-Maldonado et al. [32] reported higher activity for different leaves of *M. oleifera*: from 2436.3 to 3749.39 mg GAE/100 g. Ming-Chih et al. [33] evaluated the effect of different plant parts (leaf, stem, and stalk) and seasons (summer and winter) on the chemical composition and antioxidant activity of methanolic extracts of *M. oleifera*. Their results showed a robust scavenging effect of DPPH radicals and reducing power. The trend of the antioxidative activity as a function of *M. oleifera* plant parts was: Leaf > stem > stalk for samples from both seasons investigated. This may contribute to explain why there are so much differences in the results of multiple investigations carried out with extracts of *M. oleifera*.

The ethanol extract of *M. oleifera* showed a concentration of 4048.5 μg/mL ascorbic acid which determined a high inhibition percentage. A similar result was reported by Siddhuraju et al. [34] with extracts from freeze-dried *M. oleifera* leaves at 70% with ethanol and 80% with methanol. Therefore, identifying natural antioxidants generates evidence for protective, antiviral, antifungal, and antibacterial activities with future agronomical applications. In the present study, ethanolic extracts contained some phytochemicals such as alkaloids, soluble starch, sugar reducers, carbohydrates, carotenoids, coumarins, flavonoids, cyanogenic glycosides, quinones, saponins, and tannins. Vinoth et al. [29] found similar phytochemical compounds on *M. oleifera* ethanolic extract like flavonoids, tannins, and glycosides. However, these authors reported an absence for alkaloids, sugar reducers, and saponins, which could be due to differences in the plant extraction techniques. Packialakshmi et al. [35] reported similar bioactive components like alkaloids, flavonoids, steroids, carbohydrates, glycosides, lignin, saponins, tannins, fats and oils, phenols, amino acids and proteins, gums and mucilage in *M. oleifera*. This information is relevant for future agronomic applications. The presence of flavonoids, tannins, carotenoids, and phenols indicate that these compounds act as a primary antioxidant or free radical scavenger. Tannins have demonstrated effects as antiviral, antiparasitic, and antibacterial compounds. Furthermore, saponins are known for disrupting cell membrane activity [31]. Different compositions of phytochemicals can be due to many variables like (1) Among the rainy season and collection, (2) The origin of the plant, (3) Plant parts used for research, and (4) The extraction process. However, most of *M. oleifera* research resulted in multiple applications in different areas. Currently, there are few reports in the agronomic field, so ouranalysis presents some basis for its use in this field.

The results of the IR analysis also revealed that the components of *M. oleifera* could be aliphatic or aromatic. It may be inferred that aromatic or aliphatic alcohols or phenols, amines, ketones, esters, carboxylic acids, and some nitrogen's compounds are some constituents of the *M. oleifera* ethanolic extract. Shanmugavel et al. [31] reported similar groups to those found in our research to indicate the presence of bio-actives such as phenols and flavonoids in the range of 3387.33 cm^{-1} assigned to an alcohol, and hydroxyl group (N-H, O-H), on the fields 2931.66 cm^{-1}. Packialakshmi et al. [35] reported similar groups on *M. oleifera* up to areas of alcohol, methyl, and alkyl groups. However, because the spectrum is from an extract, the fingerprint region cannot be particularly assigned to any specific molecule.

In conclusion, the results demonstrated the potential of bio-actives from *M. oleifera* as possible biological control of *P. syringae pv. tomato*, *X. axonopodis*, *C. michiganensis* ssp. *michiganensis*, *R. solanacearum*, and *A. tumefeciens*. Although more studies are required, such as under field and greenhouse conditions, the ethanol extract of *M. oleifera* leaves can be a strong candidate for replacing synthetic bactericides. *Moringa oleifera* extract showed antibacterial and antioxidant capacities. It also confirmed high values for TFC, TPC, ACE, and different families or groups of phytochemical families of compounds. On the other hand, it will be necessary to compare *M. oleifera* with different plant origins or species. This will allow to compare the efficiency that they may present for future applications in the agronomic sector.

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