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RESEARCH PAPER

Antibacterial Activity of Moroccan Saffron By-product Extracts against *Clavibacter michiganensis subsp. michiganensis*: The Causal Agent of Tomato's Bacterial Canker

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النشاط المضاد للبكتيريا لمستخلصات الزعفران المغربي الثانوية ضد *Clavibacter michiganensis subsp. michiganensis*: العامل المسبب لقرحة الطماطم البكتيرية

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Abstract. This study aimed to investigate the effect of Moroccan saffron (*Crocus sativus*) floral by-products on the causal agent of bacterial canker of tomato caused by *Clavibacter Michiganesis subsp Michiganesis* (*CMM*). This work intends to replace chemical treatment methods with ecological, safe, and less expensive methods using extracts of saffron by-products. The phytochemical screening and the antioxidant activity of saffron by-products were studied. Moreover, the antibacterial activity of ethanolic extracts of saffron by-products was determined using the agar well diffusion method. The minimum inhibitory concentrations (MIC) of the saffron by-product extracts were determined by the dilution method using 96-well microplates. The results showed that the ethanolic extracts of saffron by-products constitute a significant source of bioactive molecules endowed with antioxidant activity. Further, all the tested saffron extracts inhibited the bacterial growth of (*CMM*) with an inhibition zone diameter ranging from 3 to 31 mm. Thus, this study aimed to valorize the saffron by-products generated after the harvest of the stigmas, in the biological treatment of tomato diseases.

Keywords: Saffron by-products, biopesticide, tomato, bacterial canker, *Clavibacter michiganensis subsp michiganensis*

الخلاصة: تهدف هذه الدراسة إلى دراسة تأثير المنتجات الثانوية لزهرة الزعفران المغربي (*Crocus sativus*) على العامل المسبب للتقرح البكتيري على الطماطم المسبب عن بكتيريا *CMM* (*Clavibacter michiganesis subsp michiganesis*). يهدف هذا العمل إلى استبدال طرق المعالجة الكيميائية بطرق بيئية وآمنة وأقل تكلفة باستخدام مستخلصات منتجات الزعفران الثانوية. تمت دراسة الفحص الكيميائي النباتي والنشاط المضاد للأكسدة لمنتجات الزعفران الثانوية. علاوة على ذلك، تم تحديد النشاط المضاد للبكتيريا للمستخلصات لمنتجات الزعفران الثانوية باستخدام طريقة انتشار الآجار. تم تحديد التركيزات المانعة الدنيا (MIC) لمستخلصات منتجات الزعفران الثانوية بواسطة طريقة التخفيف. أظهرت النتائج أن مستخلصات منتجات الزعفران الثانوية تشكل مصدرا هاما للجزيئات النشطة بيولوجيا والتي تتمتع بنشاط مضاد للأكسدة مهم. علاوة على ذلك، أدت جميع مستخلصات الزعفران المختبرة إلى منع النمو البكتيري لـ (*CMM*) حيث تراوح قطر منطقة المنع من ٣ إلى ٣١ ملمتر. تهدف هذه الدراسة إلى تثمين منتجات الزعفران الثانوية الناتجة بعد حصاد الوصمات في المعالجة البيولوجية لأمراض الطماطم

الكلمات الرئيسية: منتجات الزعفران الثانوية، المبيدات الحيوية، الطماطم، التقرح البكتيري، *Clavibacter michiganensis subsp michiganensis*

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Introduction

Pesticides used in a growing area are intended to prevent potential crop losses and poor products. Nevertheless, chemical pesticides are destructive to the environment, affecting the surface and groundwater quality soil health, and reducing aquatic and terrestrial species (Rani et al., 2021). However, naturally occurring pesticides are less toxic and safer than chemical pesticides due to their high biodegradability (Agboola et al., 2022). Several natural substances have been used for the production of bio-pesticides including plants, microbes, and nanoparticles (Ayilara et al., 2023). Due to their varying levels of secondary metabolites, plant extracts, and their essential oils have been widely used as biopesticides for controlling various agricultural crop diseases (Khursheed et al., 2022).

Morocco is listed among the top 15 tomato-producing countries in Africa (Isaac Kojo Arah et al., 2015). In 2019, tomato production in Africa was estimated at 6731.22 million tons (Ofori et al., 2022). However, the tomato crop is frequently affected by several diseases that are caused by various kinds of microorganisms (Chohan et al., 2017). The most destructive diseases in tomato production is the bacterial canker of tomatoes caused by *Clavibacter michiganensis subsp. michiganensis (CMM)* (Peritore-Galve et al., 2021). The phytopathogenic agent is symptomatic on the leaves (wilting), on the fruit (spots) and stem (canker) (Gartemann et al., 2003). However, the use of chemical pesticide in tomato cultivation appears to be dangerous for users. Several health risks are due to pesticide exposure, such as headaches, skin burns, dizziness, itching, muscle and abdominal pain (Hernández and Aguilar, 2023). Furthermore, scientific research focuses on using various biological sources, such as microorganisms, that present an effective option for the control of plant diseases avoiding thereby health and environmental risks (Anusha et al., 2019). Besides, nanoparticles had been used as biopesticides against plant pathogenic bacteria. Copper, silver, selenium and other metallic nanoparticles have been investigated against *CMM* (Perfileva et al., 2018; Cumplido-Nájera et al., 2019; Vera-Reyes et al., 2019). Furthermore, plant extracts and plant essential oils have been extensively studied. (Amkraz et al., 2014) investigated the impact of aqueous extracts of several plant species harvested from the Souss-Massa Draa region in Morocco, on the bacterial agent *CMM*. The antibacterial potential and the content of secondary metabolites in the different plants tested had been positively correlated.

Crocus sativus, also known as saffron, is one of the traditional medicinal and aromatic plants known for its antioxidant and antidepressant properties (Mzabri et al., 2019). The main area of saffron cultivation in Morocco is located in the Taliouine and Taznakht regions. Taliouine region, that is belong to the province of Taroudant in the Souss Massa region, is characterized by a continental climate with an annual average rainfall of 119.5 mm and a temperature with an annual average of 14.7°C (Dubois, 2010). Several communities which produce much more saffron in Taliouine regions are illustrated in Figure 1. However, the pruning operation of saffron stigmas produces an important quantity of floral residues such as petals, stamens and leaves (Dubois, 2010). However, these by-products contain various chemical compounds such as phenols, flavonoids, anthocyanins, etc. (Jadouali et al., 2018). Furthermore, saffron by-products have excellent antimicrobial activity against different bacteria and fungi including foodborne pathogens (Kakouri et al., 2017; Zara et al., 2021).

In this regard, the present study aimed to assess the effect of Moroccan saffron (*Crocus sativus*) floral by-products on the causal agent of bacterial canker of tomato (*CMM*). Herein, we analyzed the chemical profile of ethanolic extracts of saffron by-products namely, phenolic, flavonoids, and anthocyanins content. Moreover, we tested, for the first time, the antimicrobial activities of saffron by-product extracts against the tomato bacterial canker caused by *CMM*.

Materials and Methods

Plant Materials Collection

Saffron by-products were collected during the period of saffron harvest, (November 2022) in the Askaouen farmers in the Taliouine region, Province of Taroudant, Morocco. The pruned flowers of saffron have been separated manually in the laboratory. Saffron floral parts (petals, stamens, and pruned flowers) were dried in an oven at 30°C for 3 h. Then, the samples were crushed and placed in clean glass bottles and separated as petals, stamens and pruned flowers as indicated in Figure 1A.

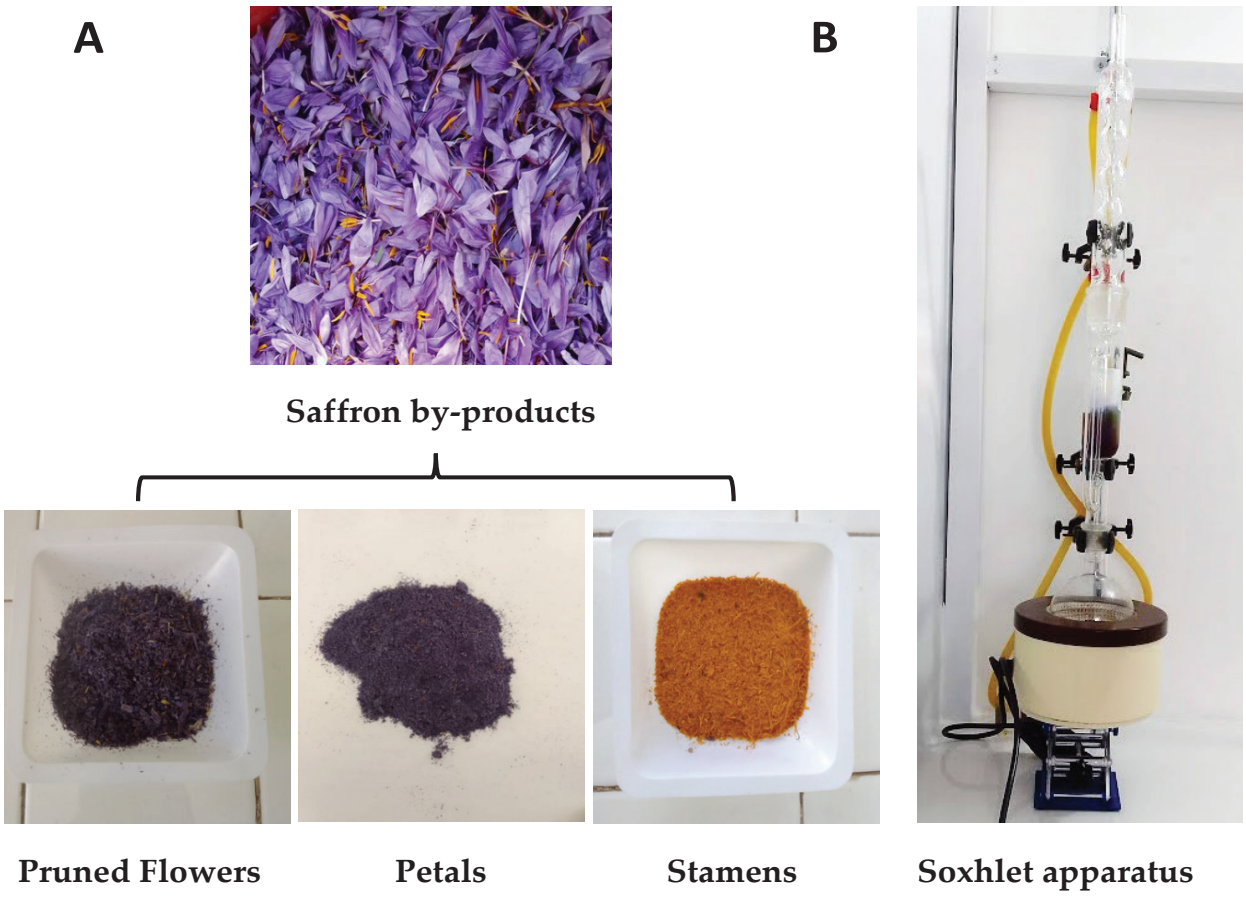
Plant Extracts Preparation

Hot extraction was performed using a soxhlet system. In Brief, 10 g of each part (petal, stamens and pruned flower) were placed in the cellulose cartridge, then 200 ml ethanol was filled into a flask and heated to boiling using a heating mantle. Soxhlet apparatus is illustrated in the Figure 1B. Then, the ethanolic extracts were centrifuged twice at 2000 rpm for 10 min to remove any form of impurities. The solvent has been removed under vacuum. The concentrated ethanolic extracts were stored at 4°C and subsequently screened for phytochemical substances.

Phytochemical Screening of Saffron by-product Extracts

Total Phenolic Content (TPC): Polyphenols *content in saffron by-products (petals, stamens and pruned flowers) was determined using Folin Ciocalteu method (*Tajik *et al.,* 2019*). Extracts were mixed with the Folin-Ciocalteu (10%; v/v)*

Figure 1. Saffron by-products preparation (a), and extraction apparatus (b)



reagent and sodium carbonate (Na_2CO_3) solution (7.5%). After 2 h in the dark, the absorbance of each sample was measured by a UV-vis spectrophotometer at 765 nm. Gallic acid was used as a standard to draw the calibration curve.

Total Flavonoids Content (TFC): Flavonoids content in saffron by-products was determined using the Aluminium chloride colorimetric method (Tajik et al., 2019). In this method, each ethanolic extract (0.5 mL) was mixed with Aluminium chloride (100 µl, 10%; (v/v). Samples were diluted using ethanol. Absorbances were measured after 30 min in the dark at 415 nm using a UV-visible spectrophotometer. Rutin was used as a standard.

Total Anthocyanin Content (TAC): The measurement of the total content of anthocyanins was carried out for the ethanolic extracts of two samples: petals and pruned flowers, using the pH difference method (Khazaei et al., 2016). Two buffer solutions were used for the pH change: (KCl, 0.025 M, pH 1) and (CH_3COONa, 0.4 M, pH 4.5). Briefly, 0.5 mL of each extract was mixed with 2.5 mL of each buffer solution. Absorbances were measured at 520 nm and 700 nm by a UV-Vis spectrophotometer after 15 min in the dark. The total anthocyanins content (TAC) was determined by the following formula:

$$TAC = \frac{A * MW * DF * 1000}{\varepsilon * b} \quad (1)$$

A = [(Abs 520nm – Abs 700nm) $pH_{=1}$] - [(Abs 520nm – Abs 700nm) $pH_{=4.5}$], MW: molecular weight of delphinidin 3-glucoside (500.84 g/mol), DF: Dilution factor, ε: Molar absorptivity, b: Thickness of the cuvette (1 cm)

Antioxidant Activity

The measurement of the antioxidant activity was carried out using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH method) (Idris et al., 2022). Briefly, the DPPH solution (1.5 mL, $6x10^{-5}$M) was mixed with 50 µL of each extract. The absorbance was measured at 515 nm using a UV-visible spectrophotometer. Ascorbic acid was the positive control. The inhibition percentage of DPPH was determined using the following formula:

$$Inhibition\ (\%) = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} * 100 \quad (2)$$

Antibacterial Activity

Well Diffusion Test: Clavibacter michiganensis subsp. michiganensis was provided by the microbiology laboratory of the Regional Centre of Agricultural Research of Agadir, National Institute of Agriculture Research, Inezgane, Agadir, Morocco. The agar well diffusion method was used to evaluate the antibacterial activity of ethanolic extracts of saffron by-products. After activation of CMM in Nutrient Broth Yeast (NBY) agar medium. The optical density of the bacterial suspension was adjusted to 0.7 at 600 nm (which is equivalent to a concentration of 10^8 CFU mL^{-1}) (Aksoy et al., 2021). Three concentrations have been prepared using ethanol 30 % as a dilution solvent (3 mg/mL, 20 mg/mL and 50 mg/mL). Thereafter, 100 µL of the bacterial suspension was spread on the surface of the culture medium. 50 µL of each concentration of the prepared extracts was placed into the wells. After 2h of pre-incubation at 4°C, the dishes were incubated at 29°C for 48 hours. Ethanol (30%) was used as a negative control. The diameter of the inhibition zones around the wells were measured with a ruler.

Minimum Inhibitory Concentration: The minimum inhibitory concentrations (MIC) of the saffron by-product extracts were determined using the dilution method using 96-well microplates. Indeed, the wells were filled by 100 µL of NBY media. The concentration of the initial stock solution (SS) of each extract (PF: Pruned Flower, P: Petals, S: Stamens) was 50 mg/mL. The bacterial suspension was adjusted to 10^6 CFU/mL (Mastrogiovanni et al., 2011). The wells in the first vertical line are filled with

200µl of the stock solution (SS) of each extract. The wells in the last vertical line (numbered 12) are filled with 200µl of the10^6 CFU/mL bacterial suspension as a control for bacterial growth, and the wells in the vertical line (numbered 11) are filled with 200µl of NBY broth as a sterility control. A successive dilution series was carried out to have final concentrations ranging from (25 mg/mL to 0.0977 mg/mL). Finally, the microplate was incubated at 29°C for 48 h.

Statistical Analysis

All experimental tests were carried out in triplicate. Data were subjected to analysis of variance and the means were compared by the Tukey test ($P < 0.05$) using IBM SPSS Statistics version.21.

Results

Phytochemical Screening of Ethanolic Extract of Saffron By-Products

The total phenolic, flavonoid, and anthocyanin content of the ethanolic extracts of saffron by-products are shown in Table 1. The ethanolic extracts of the petals and pruned flowers of saffron have a high content of anthocyanins with 15.8 ± 0.8 mg Delp/g DW of extract and 14.1±1.2 mg Delp/g DW of extract, respectively. While the saffron stamens contain a low content of anthocyanins: 2.41 ± 0.99 mg Delp/g DW of extract. Besides, the total flavonoid content in the three saffron parts tested is higher than the total polyphenols content. For example, the flavonoids content of saffron petals was 4.91 ± 0.14 mg RE/g DW, while their polyphenols content was only 1.10 ± 0.11 mg GAE/g DW.

Antioxidant Activity of Saffron by-Product Extracts

The antioxidant activity of ethanolic extracts of saffron by-products is presented in Table 2. The results show that the concentration of ethanolic extracts of saffron by-products influences their antioxidant activity. As the concentration of saffron by-product extracts increased, their antioxidant potential also increased. The highest inhibition percentage was attributed to saffron petal extracts (68.93% ± 0.81), followed by saffron flower extracts (69.79% ± 0.59) and saffron stamen extracts (71.10% ± 0.59). Moreover, the highest antioxidant activity is attributed to ethanolic extracts of saffron petals with an IC50 value of 5.48 ± 0.31 mg/mL, followed by ethanolic extract of stamens with an IC50 of 5.64 ± 0.21 mg/mL, and then of the pruned flower with an IC50 of 6.13 ± 0.12 mg/mL. Therefore, the saffron petal extracts present high antioxidant activity compared to other extracts of saffron by-products.

Table 1. Phytochemical screening of ethanolic extracts of saffron by-products

Samples	Total Polyphenols[1]	Total Flavonoids[2]	Total Anthocyanins[3]
Petals	1.10^{a} ± 0.11	4.91^{a} ± 0.14	15.77^{a} ± 0.78
Stamens	2.48^{a} ± 0.90	2.56^{c} ± 0.17	2.41^{b} ± 0.99
Pruned flowers	1.38^{a} ± 0.32	3.90^{b} ± 0.99	14.13^{a} ± 1.16

All results are expressed as the mean ± SD of three replicates.

Significant statistical differences ($p < 0.05$) are marked by small letters

[1]mg GAE/g DW: mg of Gallic Acid Equivalent per gram of dry weight.

[2]mg RE/g DW: mg of Routine Equivalent per gram of dry weight.

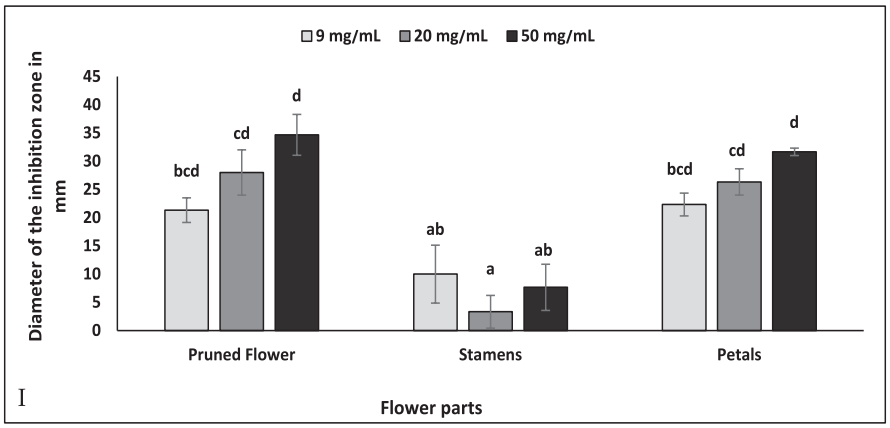
[3]mg Delp/g DW: mg of Delphinidin 3-glucoside per gram of dry weight.

Antibacterial Activity of Saffron By-Product Extracts

The inhibition zone diameters of saffron by-product extracts against *CMM* are represented graphically in Figure 3.I, while the petri dishes images of the antibacterial test are shown in Figure 3. II. The results showed that the diameters of the zones of inhibition increase as the concentrations of extracts of saffron petals and pruned flowers increase from 9 mg/mL to 50 mg/mL. The highest inhibition was attributed to pruned flowers extract (34.67 mm ± 7.62), followed by petals extract (31.67 mm ± 0.66) at 50 mg/mL, respectively. While saffron stamens extracts showed a high inhibition zone dimeter (10 mm ± 5.13) at a low concentration of 9mg/mL, while at 50 mg/mL the inhibition zone dimeter was 7.66 mm ± 4.09.

On the other hand, the minimum inhibitory concentrations (the lowest concentration of a substance that can inhibit the growth of a microorganism) of ethanolic extract of saffron by-products are shown in Figure 4; the color indicator was used to reveal bacterial growth in each well. The red color indicates the presence of *CMM*, while the other colors indicate the absence of CMM. The three lines on the microplate indicate that the antibacterial test was performed in triplicate. The results show that wells numbered 6 (PF), 2 (S), and 4 (P) present the minimum concentrations required for inhibition of CMM growth. Table 3 presents the concentrations of ethanolic extracts of saffron by-products containing in each plate well. In fact, the ethanolic extracts of the saffron pruned flower (PF) showed a maximum antimicrobial effect against *CMM* with a minimal inhibitory concentration of 1.56 mg/mL. While the minimal inhibitory concentration of ethanolic extracts of saffron petals (P) was 6.25 mg/mL. The lowest antimicrobial effect against *CMM* was attributed to the ethanolic extract of saffron stamens, with a MIC value of 25 mg/mL.

Figure 2. Graphical representation of the zone inhibition of saffron by-product extracts against CMM (I), and petri dishes images of saffron pruned flowers (A), petals (B) and stamens (C) extract against CMM at different concentrations: 1 (3mg/ml), 2 (20 mg/ml) and 3 (50 mg/ml).



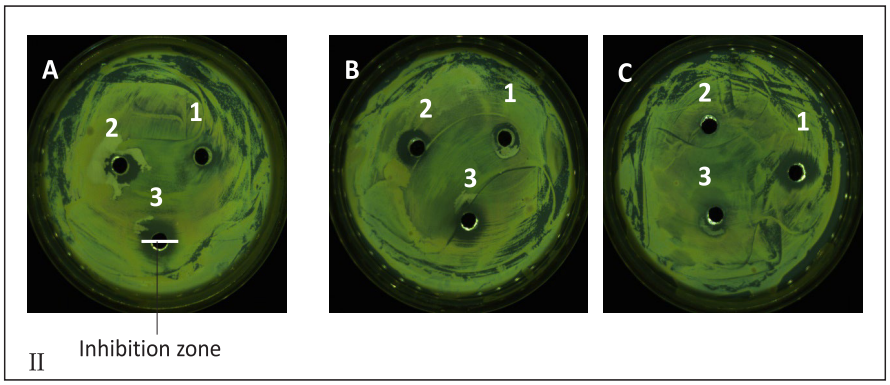


Figure 3. Minimal Inhibitory Concentration of saffron by-products extract against CMM (PF: Pruned Flower; P: Petals; S: Stamens; SS: Stock Solution)

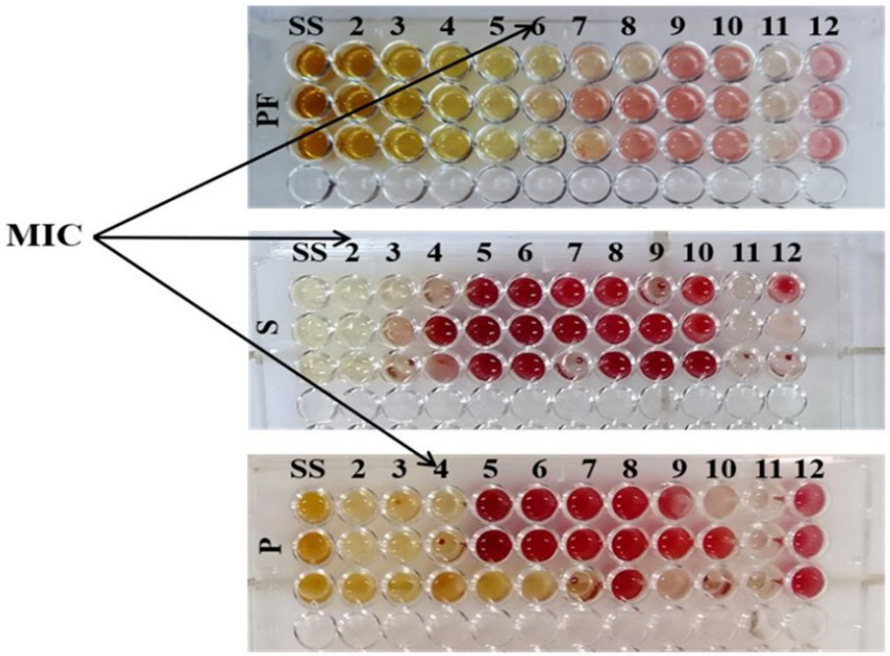


Table 2. Inhibition percentage and IC50 of ethanolic extract of saffron by-products

	% of inhibition of ethanolic extract of saffron by-products		
Concentration mg/mL	Petals	Stamens	Pruned flowers
1.66	$21.96^{ab} \pm 3.01$	$19.51^{ab} \pm 1.86$	$16.80^{a} \pm 1.45$
6.66	$23.59^{ab} \pm 4.47$	$18.75^{ab} \pm 1.00$	$16.16^{a} \pm 0.43$
13.33	$37.00^{b} \pm 10.87$	$20.33^{ab} \pm 1.14$	$15.03^{a} \pm 1.11$
16.66	$25.49^{ab} \pm 4.64$	$21.42^{ab} \pm 1.31$	$16.84^{a} \pm 1.15$
25.00	$29.30^{ab} \pm 6.54$	$22.50^{ab} \pm 1.25$	$21.05^{ab} \pm 2.29$
33.33	$31.83^{ab} \pm 7.65$	$23.73^{ab} \pm 0.60$	$21.73^{ab} \pm 1.97$
50.00	$68.93^{c} \pm 0.81$	$71.10^{c} \pm 2.53$	$69.79^{c} \pm 0.59$
IC 50 mg/mL	$5.48^{a} \pm 0.31$	$5.64^{a} \pm 0.21$	$6.13^{a} \pm 0.12$

All results are expressed as the mean ± SD of three replicates.
Significant statistical differences ($p < 0.05$) are marked by small letters.

Table 3. Concentrations of ethanolic extracts of saffron by-products containing in each well

Well number	Concentration (mg/mL)
SS*	50
2	25
3	12.5
4	6.25
5	3.125
6	1.5625
7	0.7813
8	0.3906
9	0.1953
10	0.0977
11	NBY*
12	BS*

SS*: Stock Solution; NBY*: Nutrient Broth Yeast; BS*: Bacterial Suspension.

Discussion

Our study aimed to replace chemical pesticides, especially those used to treat bacterial canker of tomatoes caused by *CMM*, with ecological, safe and less costly methods using extracts of saffron by-products. The phytochemical screening and antioxidant activity study of saffron byproducts were conducted to improve the study and explain the reason for antibacterial activity of extracts. Saffron flower, petal and stamen extracts were tested against the growth of *CMM*. Results showed that the highest inhibition was observed with the pruned flower extract (34.67 mm), followed by the petal extract (31.67 mm), then saffron stamens (7,66 mm) at a concentration of 50 mg/mL. In a similar study, 90% of all the plants harvested in southern Morocco had a significant inhibition activity against *CMM.* The best inhibition zone diameter was obtained with *Lavandula coronopifolia* (4.88 cm) due to its bioactive compounds, especially the linalool and acetate of linalyl (Talibi et al., 2014). Furthermore, among seven plant harvested in Pakistan, *Peganum harmala* showed the maximum inhibition of *CMM* growth (14.40 mm), due to its hight content of alkaloids including harmine which has a powerful antibacterial effect against pathogens (Siddique et al., 2020).

In contrast, saffron pruned flowers extract (PF) showed maximum antibacterial activity against *CMM* with a MIC of 1.56 mg/mL, followed by saffron petal (P) extracts with a MIC of 6.25 mg/mL. The extract of saffron stamens with a MIC of 25 mg/mL was found to have the lowest antimicrobial effect against CMM. The comparative study of the antimicrobial activity of saffron petals and stamens against food borne pathogens was carried out by (Asgarpanah et al., 2013). The MIC of methanolic extracts of saffron petals were higher than those of saffron stamens. Furthermore, saffron petal extracts showed strong inhibitory activity with MIC of 15.63 µg/mL against the gram-negative bacteria *Escherichia coli* (ATCC 25922), and the fungi strain *Candida albicans* (Wali et al., 2020). Moreover, saffron leaf extracts were effective against the growth of *Staphylococcus aureus* with a MIC of 6.5 mg/mL (Ökmen et al., 2016).

The mechanism of action behind the inhibition of bacterial growth is currently under discussion. Generally, bacterial growth could be inhibited through several actions that target various cellular structures (Zheng et al., 2020). The benzoic acid, hydroxy-methyl-furfurole, anionic acid, nicotine and sterol compounds contained in the methanolic extract of *Nicotiana plumbaginifolia* leaf are responsible for their antibacterial effect against plant pathogenic bacteria (Javaheri et al., 2022). Moreover, the growth of plant pathogens can be inhibited by a group of antimicrobial peptides, so called bacteriocins, produced by bacteria and plants (Mirzaee et al., 2021).

Concerning the phytochemical screening of extracts of saffron by-products, the extracts of saffron petals and pruned flowers are rich in anthocyanins (15.77 and 14.13 Delp/g DW extract,

respectively). Saffron stamens, on the other hand, are low in anthocyanins (2.41 mg Delp/g DW extract). In addition, flavonoid compounds in the three saffron parts tested were more dominant than polyphenolic compounds.

Our results are similar to those reported by (Jadouali et al., 2017), the ethanolic extract of saffron petals contains a flavonoid content of 42.56 mg CE.g^{-1} dry weight and a polyphenol content of 30.26 mg GAE.mg^{-1} dry weight. Moreover, (Turcov et al., 2022) reported that the saffron waste extracts obtained by heat reflux extraction contained significantly more flavonoids (197.84 mg QE/g) than phenolics compounds (29.47 µg GAE/g). Similarly, the saffron stamen samples contain three major components of flavanol glycosides, namely quercetin-3-O-sophoroside, kaempferol-3-O-sophoroside, and kaempferol-3-O-glucoside (Mottaghipisheh et al., 2020).

Food colors are determined by the presence of several types of pigments, including chlorophyll (green), carotenoids (yellow-orange) and anthocyanins (red-purple) (Kutlu et al., 2022). These pigments are endowed with divers therapeutic, antioxidant and antimicrobial properties (Molina et al., 2023). Several studies have been carried out on the extraction and phytochemical analyses of various medicinal and aromatic plants in order to assess the enormous effects of their active compounds (Nortjie et al., 2022).

With regard to the antioxidant study of saffron by-product extracts, the highest inhibition percentage was attributed to saffron petal extracts (68.93%), followed by saffron flower extracts (69.79%) and saffron stamen extracts (71.10%). Various plant parts such as leaves, fruits, flowers, roots etc. are considered as rich sources of antioxidants. The chemical compounds present in these biological products contain several phytochemicals component that have various functional groups, including hydroxyl groups (OH), aromatic rings (CH), carbonyl and carboxyl groups (CO) (Waisundara, 2021). Flavonoids act as electron donors to the DPPH molecule. This reaction produces unstable radical species of flavonoids and DPPH, to restore their stable state, the radical flavonoids are converted into a stable quinoid structure, and the radical DPPH forms a stable yellow form (Warsi and Sholichah, 2017). Thus, in our study, the high content of flavonoids in saffron stamens and petals explains their high antioxidant activity (Table 1 and 2).

According to these results, ethanolic extracts of the saffron pruned flower, which combines two parts (stamens and petals), inhibit the growth of *CMM* with at a higher level than ethanolic extracts of the separate petals and stamens. Thus, the synergistic effect of all the bioactive compounds present in the different parts of saffron conferred significant antimicrobial activity on the by-products of this plant (Rahaiee et al., 2015) . Some combinations between natural substances, and other antimicrobial agents such as antibiotics, have been demonstrated to have a synergistic effect towards the inhibition of bacterial growth (Regueira et al., 2017). It can be concluded that the direct use of the pruned saffron flower obtained after cutting the stigmas, without going back to separating the petals and stamens, remains more effective in obtaining satisfactory results in inhibiting the proliferation of the causal organism of tomato bacterial canker. These results appear to be very useful and practical for the development of biopesticides for the treatment of tomato crops. In a similar study, saffron by-products have been used to treat eggplant cultivation, the water extract of saffron petal was regularly used as a foliar spray (Khoulati et al., 2020). Besides, the aqueous extracts of cut flowers, petals and stamens of saffron have been studied for germination treatment of winter durum wheat seeds (Mikolajchuk et al., 2023).

Conclusion

Saffron by-products present a very rich source of active compounds. Due to their chemical profile, the application of saffron by-products has been extensively investigated as antimicrobial agents for the control of plant diseases. This study aims to evaluate the antibacterial effect of the diffe-

rent floral parts of saffron, in particular petals, stamens, and pruned flowers against *Clavibacter michiganensis subsp michiganensis*, the tomato canker's pathogen. Following the results of this study, saffron by-products contain a remarkable and important content of polyphenols, flavonoids, and anthocyanins. The flavonoid content was higher in the stamens. The pruned flowers contained a high content of polyphenols while the anthocyanin content was higher in the petals. Furthermore, saffron by-products have a significant antioxidant activity with an IC50 ranging from 5.48 mg/mL to 6.13 mg/mL. However, there was not a considerable difference between the antioxidant potential of the saffron by-products. Regarding antimicrobial activity, the inhibitory activity of saffron pruned flower against *CMM* was higher than that of petals and stamens. The diameters of the inhibition zones of saffron by-product extracts range from 3 to 34 mm. The diameter of the inhibition zone of saffron pruned flower, and petal extracts increases with increasing extract concentrations. These results were approved by the MIC test, in which the MIC of saffron pruned flowers was lower than that of the stamens and petals. Hence, the synergistic effect of all the bioactive components contained in the stamens and petals of saffron has conferred on its pruned flower an important antioxidant and antimicrobial activity against the causal agent of tomato canker: *CMM*. From the following results, it can be concluded that saffron by-products present a potential biopesticide to protect tomato crops.

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Declaration of competing interest

Authors declare that they have no competing financial interests or personal relationships that could appear to have influenced the work reported in this paper.

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