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Anti-Quorum Sensing Effects of Some Medicinal and Aromatic Plant Extracts on *Xanthomonas axonopodis* pv. *Phaseoli*[#]

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ABSTRACT

Xanthomonas axonopodis pv. *phaseoli* (*Xap*) is known as one of the most important seed-borne destructive pathogens on beans worldwide. Nowadays, *Xap* is considered to acquire resistance to antibiotics and synthetic bactericides which concerns the scientific world for its future management. This has made the use of plant extracts, the best alternative in the control of plant disease pathogens by inhibiting the quorum sensing (QS) mediated virulence factors. This research was designed to investigate the antibacterial activities and the anti-QS effects of the 14 different aromatic and medicinal plants against QS-mediated virulence factors of *Xap*. The results revealed that *Syzgium aromaticum* showed the largest inhibition zone diameter and strongest antimicrobial (antibacterial) effect among the 14 plant extracts followed by *Thymus vulgaris* and *Coriandrum sativum*. Similarly, the lowest swarming, swimming, and twitching motility values were measured from the *Syzgium aromaticum* application followed by *Coriandrum sativum*, *Thymus vulgaris*, *Brassica nigra*, *Lepidium sativum*, and *Ruta chalepenis*. These results indicated that *S. aromaticum*, *C. sativum*, *T. vulgaris*, *B. nigra*, *L. sativum*, and *R. chalepenis* will be a potential candidates as anti-quorum sensing agents in preventing common bacterial disease of beans caused by *Xap*. Compounds derived from aromatic and medicinal plants have demonstrated successful control of diseases in crops and the use of these substances provides a valuable tool to the growers around the world for diseases management in organic production.

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Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the main produced and consumed pulse crops worldwide (FAO, 2016) and its yield and seed quality are significantly decreased mainly due to common bacterial blight (CBB). According to Zaumeyer and Thomas (1957), CBB which is caused by Xap is a destructive disease of common bean worldwide. Since there is no satisfactory chemical control against Xap, biological control methods and the use of resistant varieties are important management strategies (Zapata, 1997).

Recently, the Quorum Sensing (QS) mechanism has become a new strategic target to combat pathogenic bacteria as a biological control mechanism, due to its main role in bacterial virulence and pro-survival effect on bacterial cells in biofilms (Yüzbaşıoğlu et al., 2018). The word QS is broadly defined as a cell-to-cell communication of bacteria through chemical language. The subsequent discovery of compounds that inhibit QS, named anti-QS agents could provide a new method of combating bacterial disease. The hazardous effect of the widely used synthetic chemicals to the environment and to human health makes the use of biologically active products (plant extracts and essential oils), an attractive alternative in the control of bacterial pathogens (Burt, 2004; Lo Cantore et al., 2009). QS systems have been shown to be inhibited by bioactive molecules produced by many aromatic and medicinal plants (Novick and Geisinger, 2008).

In the study of Adonizio et al., (2006) in South Florida, 50 medicinal plants were characterized for anti-QS activity using *Chromobacterium violaceum* and *Agrobacterium tumefaciens* as a bio-monitor strain. Of these plants, *Chamaecyce hypericifolia*, *Conocarpus erectus*, *Bucida burceras*, *Callistemon viminalis*, *Tetrazygia bicolor*, and *Quercus virginiana* showed QS inhibition activity. In another study, Mary and Banu (2015), the methanolic leaf extract obtained from *Vitex trifolia* showed anti quorum sensing activity in *Pseudomonas aeruginosa*. Al-Haidari et al. (2016) also reported that the extracts of some medicinal plants like *Allium cepa*, *Laurus nobilis*, *Citrus sinensis*, *Coriandrum sativum*, and *Elettaria cardamomum*, exhibited a potent quorum quenching effect.

Crude extracts from *Epilobium angustifolium*, *Tanacetum balsamita*, *Quercus robur* and *Quercus frainetto* showed anti-QS activity and significantly reduced violacein production in *C. violaceum* (Yüzbaşıoğlu et al., 2018). Anti-QS has also been demonstrated for plants originated from different regions including Europe (Tolmacheva et al., 2014), Korea (Damte et al., 2013), Iran (Mahmoudi et al., 2014), China (Koh and Tham, 2011) and India (Zahin et al., 2010). Similarly, in Ta et al. (2014) study, twelve of seventy-one species collected from neotropical rainforests in Costa Rica were shown to possess significant anti-QS activities. Fruits such as blueberry, raspberry, cranberry, blackberry, and grape, and herbs including ginger, thyme, basil, oregano, kale and turmeric exhibited moderate inhibition of QS-controlled processes on *C. violaceum* bio-monitor strain and the swarming motility of *E. coli* and *P. aeruginosa* (Vattem et al., 2007). Of the 24 Indian medicinal plants screened, five of them demonstrated varying levels of violacein production in the reporter strains (Zahin et al., 2010). Likewise, Khan et al. (2009) reported that *Syzygium aromaticum* oil showed promising anti-QS activity on both wild and mutant strains followed by lavender, cinnamon, and peppermint oils. In South America, the *Minthostachys mollis* essential oil is a good candidate for the development of anti-QS products with a potential application in the control of bacterial diseases mediated by QS (Pellegrini et al., 2014).

However, there was no study about the anti-QS effects of different plant extracts on QS-mediated virulence factors in the case of Xap. Management of CBB caused by Xap now a day is a challenge due to the limited efficacy of management strategies, as the pathogen acquires resistance to antibiotics and fixed copper bactericides and host resistance has not proven durable. Therefore, there is a need to develop an eco-friendly alternative and reduced-risk disease management approach.

In this study, we designed to evaluate the antibacterial activities and the anti-QS effects of the different aromatic and medicinal plants widely used in Ethiopia on QS mediated virulence factors swarming, swimming, and twitching motility of Xap.

Materials and Methods

Plant Materials and Crude Extract Preparation

Fourteen aromatic and medicinal plants (Table 1) were collected from local open markets of Addis Ababa town and purchased from different agricultural areas of Ethiopia. The collected plants and their parts were taxonomically identified and proved at Melkasa Agricultural Research Center and Addis Ababa University assisted by plant biologist and botanist professionals.

Table 1. Aromatic and medicinal plants used in the QS experiments against *X. a.* pv. *phaseoli*

Common name	Scientific Name	Local name		Plant parts used
		Turkish	Amharic	
Cinnamon	*Cinnamon zeylanicum*	Tarçin	Kerefa	Bark
Clove	*Syzgium aromaticum*	Karanfil	Kurunfud	Flower Head
Thyme	*Thymus vulgaris*	Kekik	Tosign	Leaf
Mustard	*Brassica nigra*	Hardal	Snafich	Seed
Black cumin	*Nigella sativa* L.	Çörek Otu	Tekur Azmud	Seed
Garden cress	*Lepidium sativum*	Tere	Feto	Seed
Ethiopian Mustard	*Brassica carrinata*	Hardal	Gomenzer	Seed
Garlic	*Allium sativum*	Sarimsak	Nech shinkurt	Bulb
Turmeric	*Curcuma longa*	Zerdeçal	Erd	Rhizome
Coriander	*Coriandrum sativum*	Kişniş	Dinblal	Fruit
Black pepper	*Piper nigrum* L.	Kara Biber	Kundu berbere	Fruit
Garden Rue	*Ruta chalepenis* L.	Sedef Otu	Tena adam	Fruit
Moringa	*Moringa oleifera*	Moringa	Shiferaw	Leaf
Rosemary	*Rosmarinus officinalis*	Biberiye	Rozmary	Leaf shoot

Plant parts used for extraction were washed 2-3 times by running water and sterile distilled water, respectively. They were air-dried on a sterile blotter under shade at room temperature for 10 days (Soltani and Aliabadi, 2013). The plant parts of each aromatic and medicinal plant were ground by a grinding machine in the form of powder. Based on the procedures described by Kang et al. (2011**),** Soltani and Aliabadi (2013), Ibrahim and Abu-Salem (2014), Houshyar et al. (2014), Yavuz et al. (2017), and Ramena et al. (2018) with some modifications, 14 plant extracts were prepared as follows. Fifty grams of fine powder of each aromatic and medicinal plant part was soaked in 500 ml of methanol 99% (v/v) in a sterile container and incubated for seven days at normal temperature with manual shaking (Fig.1). Then the crude extract was filtered through Whatman filter paper and processed with Heidolph Rotary Evaporator for 40°C at 140rpm for the evaporation process. The extracts were stayed in the water bath for one day at about 40°C for complete removal of methanol. Finally, the semi-solid residues were stored aseptically in a sterilized bottle at -20°C until use.

Evaluation of Antibacterial Activities of Crude Plant Extracts Against Xap

To evaluate the antimicrobial activities of the medicinal and aromatic plant extracts against Xap isolate (145-X), the agar disc diffusion method was used as described by Chudasama and Thaker (2012), Houshyar et al. (2014), Bacha et al. (2016), and Yavuz et al. (2017) with some modifications. First a well-characterized by biochemical and molecular techniques and highly virulent isolate 145-X of Xap (92%) was grown on yeast dextrose-calcium carbonate agar (YDC) medium for 48 hr at 28°C. Then 48

hr old culture of Xap isolate was suspended in sterile deionized water, and adjusted using Bio-photometer (Eppendorph plus, OD650nm: 0.15) with an approximate concentration of 10^8CFU mL^{-1}.

A100µl bacterial suspension was evenly spread over the prepared nutrient agar (NA) petri dishes with a 9 cm diameter to get uniform growth of Xap. After 15-20 min of bacterial inoculation, six-millimeter diameter sterilized standard paper discs were prepared and placed on the surface of inoculated culture plates with the help of sterile forceps. Stock solutions of plant extracts were prepared by dissolving 100 mg of each plant extract in 1ml of 5% (v/v) Dimethyl Sulfoxide (DMSO) and 15 µl volume of each plant extract was pipetted (placed) onto the already prepared discs. Similarly, the same amount (15µl) of Gentamycin (10µg/ml) and DMSO were poured onto paper discs and were used as positive and negative controls, respectively. The Petri plates were left under room temperature in a laminar cabin for about 20 min by sealing with sterile parafilm to allow the uniform diffusion of extracts into the gar plate and then the plates were incubated at 28°C for 48 hr. Finally, the diameters of the inhibition growth zone of each test extract generated around discs were measured in millimeters (mm) to determine their antibacterial activity on Xap. The experiment was repeated twice with three replications and the results were summarized and expressed as mean ± SD values.

Minimum Inhibitory Concentration (MIC) Determination Assay

Following the procedures of Bacha et al (2016) and Ramena et al. (2018) with modifications, the lowest or minimum concentration of the plant extract required to inhibit the growth of Xap was determined by a disc diffusion method. Alike the antimicrobial activity procedures above, standard sterilized paper discs (6 mm in diameter) were prepared for MIC determination. Consequently, the initial stock solution (concentration) of plant extract (50mg/ml) was diluted in 1:2 serial dilutions. In other words, two-fold serial dilutions of each extract were prepared by dissolving 50, 25, 12.5, 6.25, 3.13, 1.56, and 0.78 mg in 1ml of 5% (v/v) DMSO. Freshly prepared 48 h of Xap culture was suspended in sterile distilled water (SDW) with a final concentration of 10^8 $CFUmL^{-1}$ and 100µl bacterial suspension was spread over the NA plate. After a few minutes of bacterial inoculation, seven sterilized paper discs were placed on the Petri dishes and tagging them as number one to seven, respectively with the above different extract concentrations (disc 1; 50 mg/ml, disc 2; 25 mg/ml, disc 3; 12.5 mg/ml, etc.). All discs were loaded with the same amount of plat extracts (15 µl) and the plates were incubated for 48 hr at 28°C. DMSO was used as a negative control. Finally, the zone of inhibition was observed and evaluated, and then the lowest concentration of plant extract (mg/ml) that prevented the visible growth of Xap was considered as the MIC. All samples were carried out in duplicates during MIC determination.

The Anti-Quorum Sensing Potentials of Crude Plant Extracts on Xap

Invitro evaluation of the anti-QS (swarming, swimming, and twitching motility inhibition) activities of the different plant extracts against Xap was conducted and the procedures were adapted from different researches (Hosseinzadeh et al., 2013; Zhang et al., 2014; Al-Haidari et al., 2016; Elmanama and Al-Refi, 2017; Singh et al., 2017a; Singh et al., 2017b).

Swarming motility assay of Xap

To conduct the swarming motility assays, the swarming medium and its plate were prepared using 0.8% (w/v) of nutrient broth, 0.5% (w/v) of glucose, and 0.5% (w/v) of Bacto agar. Each ingredient was mixed together and adjusted the pH to 7.2. The medium was sterilized at 121°C for 15 minutes and poured into the Petri plates when the medium was cooled to 50-45°C. To evaluate the swarming motility inhibition potential of each plant extract, five ml of semi-solid agar/swarming medium was mixed with 200 µl of each plant extract and poured into the prepared swarming plates as overly. The same amount (200 µl) of DMSO (5%) was poured instead of plant extracts as a negative control (Hosseinzadeh et al., 2013; Zhang et al., 2014; Al-Haidari et al., 2016; Elmanama and Al-Refi, 2017; Singh et al., 2017a; Singh et al., 2017b).

A pure colony of an overnight Xap isolate culture was selected and inoculated on the center of the swarming plate in the form of a point by using a sterile toothpick and plates were incubated at 28°C for 48hr. Finally, hazy zone of motility, the migration distance (diameter) of Xap was measured in mm from the center (the diameter of circular bacterial growth from the point of inoculation was measured) and the percent inhibition of swarming motility of Xap in the presence of plant extracts as compared to control treatment was determined as follow.

$$\%\text{ISwaM} = \frac{SwaMC - SwaME}{SwaMC} x\ 100$$

Where,

%ISwaM= percent inhibition of swarming motility,

SwaMC= swarming motility at control sample

SwaME= swarming motility at extract sample

(Hosseinzadeh et al., 2013; Zhang et al., 2014; Al-Haidari et al., 2016; Elmanama and Al-Refi, 2017; Singh et al., 2017a; Singh et al., 2017b).

Swimming and twitching motility assay

Both swimming and twitching motility tests were carried out with the same procedures of swarming test on swimming and twitching media. The swimming medium was prepared by using 1.0% tryptone, 0.5% yeast extract, and 0.3% agar, and twitching plates were prepared using 1g tryptone, 0.5g yeast extract, 0.5g NaCl, and 1g agar per 100 ml of distilled water. Both motility media were adjusted to 7.2 pH and sterilized at 121°C for 15 minutes and poured onto Petri plates. The swimming and twitching plates were allowed to dry at room temperature under a laminar cabin before being used. Alike swarming motility assay, to determine the swimming and twitching inhibition motility activities of plant extracts, five ml of semi-solid agar with 200 µl of each plant extract was well mixed and poured onto the prepared swimming and twitching plants as an overlay. As usual, DMSO was used as a negative control. An overnight Xap culture was inoculated on the center of the swimming plates by taking its colony using a sterile toothpick. However, in the case of the twitching test, the colony of Xap was planted by immersing it in the bottom of the twitching plate at the center. The plates were incubated at 28°C for 48hr (Hosseinzadeh et al., 2013; Zhang et al., 2014; Al-Haidari et al., 2016; Elmanama and

Al-Refi, 2017; Singh et al., 2017a; Singh et al., 2017b). At the end, the presence of a foggy zone of the motility (the diameter of the colony expansion) was measured and the average data are presented as means ± SD. All experiments were repeated twice and replicated three times by arranging in a completely randomized design (CRD). Reduction in swimming and twitching motility (percent inhibition of swimming and twitching motility) of Xap in the presence of plant extracts as compared to control treatment was estimated like the swarming test above.

Statistical Analysis

The data of each activity was analyzed for statistical significance using analysis of variance (ANOVA) of SAS software and mean separation was carried out using Duncan's Multiple Range Test (DMRT) at 0.99 (P≤0.01) level of confidence. For all cases, the average data are presented as means ± SD.

Results

Antibacterial Effects of Aromatic and Medicinal Plant Extract Against Xap and MIC Values of The Extracts

The antibacterial activity of aromatic and medicinal plant extracts against Xap showed that there was a highly significant difference among each extract for their inhibition zone diameter (Table 2 and Figure 1). All the extracts tested in the disc diffusion method showed an inhibition effect on the tested pathogen with different mean values (Figure 1). They showed various degrees of antibacterial effects against Xap ranging between 8-22.67 mm inhibition zone diameter. The crude extract from *S. aromaticum*, showed the strongest antibacterial effect and largest inhibition zone diameter (22.67 ±1.16 mm) among the tested plant extracts, which is almost near to the inhibition effect of the positive control gentamycin (25±1.00 mm). The second group of effective plant extracts included *T. vulgaris* (15.33 ± 0.58 mm), *C. sativum* (14.33 ± 0.57 mm), *N. sativa* (14 ±1 mm), B. nigra (13.33 ±0.57 mm), *L. sativum* (13 ±1 mm) and *R. Chalepenis* (13 ±1 mm). *C. longa, P. nigrum and A. sativum* inhibited the growth of the bacterium by 12.00 ± 0.0 mm, 11.33 ±0.58 mm, and 10.67 ±1.16 mm, respectively. Next to *C. zeylanicum* and *B. carrinata*, extracts of *R. officinalis* and*M. oleifera*hade the least antibacterial activities on Xap with 8 mm diameter of zone of growth inhibition (Figure 1).

The tested plant extracts were further serially diluted to make different concentrations and determined their MICs. Consequently, the MICs values of different plant extracts were ranged between 1.56 to 50 mg/ml. The lowest MIC value (1.56 mg/ml) was recorded from the extract *N. sativa* (Table 2). Generally, the estimated MICs values of the tested aromatic and medicinal plant extract obtained using the disc diffusion method for Xap bacteria were 1.56, 6.25, 12.5, 25, and 50 mg/ml.

The Anti-QS Activity of Aromatic and Medicinal Plant Extracts On QS Mediated Factors of Xap

The effects of aromatic and medicinal plant extract on swarming, swimming and twitching motility (mm) and their percent inhibition (%) of Xap isolate as compared to the control treatment (Table 3-5 and Fig. 2-7). The effect of plant extracts on both bacterial motility (mm) and its percent reduction (%) as compared to control treatment were shown significantly (P≤0.01) different from the control treatment and each other. All plant extracts have significantly reduced the motility of Xap isolate in swarm plates. The mean of swarming, swimming, and twitching motility was ranged from 11.67-61.00 mm, 7.33-58.67 mm, and 5.00-16.67 mm, respectively. In all motility assays, the lowest motility values were measured from the *S. aromaticum* extract (11.67 mm for swarming, 7.33 mm for swimming, and 5.00 mm for twitching motility)followed by *C. sativum, T. vulgaris, B. nigra, L. sativum* and *R. chalepenis*. However, all motility assessments (swarming, swimming, and twitching) were largely by far as compared to control plate [5% DMSO (Only Xap)] (78.33 mm for swarming, 69.33 mm for swimming, and 21.00 mm for twitching) (Table 3-5).

Table 2. Mean of antibacterial activities (inhibition zone diameter in mm) of tested aromatic and medicinal plant extracts and their MICs ($mgml^{-1}$) against Xap

Plant extracts	Inhibition zone diameter (mm)	MIC($mgml^{-1}$)
Cinnamon (*Cinnamon zeylanicum*)	9.67 ± 0.58^{hij}	12.5
Clove (*Syzgium aromaticum*)	22.67 ± 1.16^{b}	6.25
Thyme (*Thymus vulgaris*)	15.33 ± 0.58^{c}	6.25
Mustard (*Brassica nigra*)	13.33 ± 0.57^{cdef}	25
Black cumin (*Nigella sativa* L.)	14.00 ± 1.00^{cde}	1.56
Garden cress (*Lepidium sativum*)	13.00 ± 1.00^{def}	25
Ethiopian mustard (*Brassica carrinata*)	9.00 ± 0.00^{ij}	50
Garlic (*Allium sativum*)	10.67 ± 1.16^{ghi}	50
Turmeric (*Curcuma longa*)	12.00 ± 0.00^{efg}	6.25
Coriander (*Coriandrum sativum*)	14.33 ± 0.57^{cd}	25
Black pepper (*Piper nigrum* L.)	11.33 ± 0.58^{fgh}	25
Garden Rue (*Ruta chalepenis* L.)	13.00 ± 1.00^{def}	12.5
Moringa (*Moringa oleifera*)	8.00 ± 0.00^{j}	>50
Rosemary (*Rosmarinus officinalis*)	8.00 ± 0.00^{j}	>50
Gentamycin (positive control)	25.00 ± 1.00^{a}	-
5% DMSO (negative control)	0.00 ± 0.00^{k}	-

Means that do not share a common letter within a column are significantly different from each other at P≤0.05 according to DMRT.

Table 3. Swarming motility (mm) and inhibition percentage of swarming motility of Xap in the presence of some aromatic and medicinal plant crude extracts

Plant extracts	Diameter of swarming motility (mm)	Inhibition ofswarmingmotility over control (%)
Cinnamon (*Cinnamon zeylanicum*)	47.33 ±0.58de	39.56 ± 1.49gh
Clove (*Syzgium aromaticum*)	11.67 ±0.58L	85.11 ±0.75a
Thyme (*Thymus vulgaris*)	34.33 ±0.58j	56.16 ± 1.22bc
Mustard (*Brassica nigra*)	35.33 ±0.58ij	54.88 ±1.52c
Black cumin (*Nigella sativa* L.)	40.33 ±1.53g	48.52 ±1.31e
Garden cress (*Lepidium sativum*)	36.67 ±1.53hi	53.20 ± 1.17cd
Ethiopian mustard (*Brassica carrinata*)	56.67 ±0.58c	27.63 ± 2.11i
Garlic (*Allium sativum*)	49.00 ±1.00d	37.45 ± 0.24h
Turmeric (*Curcuma longa*)	61.00 ±1.00b	22.09 ± 2.77j
Coriander (*Coriandrum sativum*)	32.33 ±1.16k	58.69 ± 2.23b
Black pepper (*Piper nigrum* L.)	44.33 ±0.58f	43.38 ±1.73f
Garden Rue (*Ruta chalepenis* L.)	38.33 ±0.58h	51.04 ±1.59de
Moringa (*Moringa oleifera*)	58.33 ±1.53c	25.49 ±3.34i
Rosemary (*Rosmarinus officinalis*)	46.00 ±1.00ef	41.27 ±1.40fg
Control (Only Xap)	78.33 ±1.53a	--

Means that do not share a common letter within a column are significantly different from each other at P ≤ 0.05 according to DMRT.

Table 4. Swimming motility (mm) and inhibition percentage of swimming motility of Xap in the presence of some aromatic and medicinal plant crude extracts

Plant extracts	Swimming motility(mm)	Inhibition of swimming(%)
Cinnamon (*Cinnamon zeylanicum*)	41.67 ±0.58de	39.90 ±0.88fg
Clove (*Syzgium aromaticum*)	7.33 ± 0.58k	89.41 ± 1.03a
Thyme (*Thymus vulgaris)*	29.33 ±1.16j	57.68 ±1.77b
Mustard (*Brassica nigra*)	31.67 ±1.53i	54.33 ± 1.93c
Black cumin (*Nigella sativa* L.)	37.67 ±1.53g	45.63 ±3.19e
Garden cress (*Lepidium sativum*)	34.00 ± 1.00h	50.96 ±0.44d
Ethiopian mustard (*Brassica carrinata*)	45.33 ±0.58c	34.59 ±1.78h
Garlic (*Allium sativum*)	43.00 ±1.00d	37.97 ±1.62g
Turmeric (*Curcuma longa*)	58.00 ±1.00b	16.34 ±0.50i
Coriander (*Coriandrum sativum*)	28.33 ±1.16j	59.10 ±2.51b
Black pepper (*Piper nigrum* L.)	39.67 ±0.58f	42.76 ±2.05ef
Garden Rue (*Ruta chalepenis* L.)	33.00 ± 1.00hi	52.37 ± 2.47cd
Moringa (*Moringa oleifera*)	58.67 ±1.16b	15.38 ±0.62i
Rosemary (*Rosmarinus officinalis*)	40.33 ±0.58ef	41.81 ± 1.63f
Control (Only Xap)	69.33 ± 1.53a	

Means that do not share a common letter within a column are significantly different from each other at P≤0.05 according to DMRT.

Table 5. Twitching motility (mm) and inhibition percentage of twitching motility of Xap in the presence of some aromatic and medicinal plant crude extracts

Plant extracts	Twitching motility (mm)	Inhibition of twitching(%)
Cinnamon (*Cinnamon zeylanicum*)	16.33 ±0.58bc	22.11 ±4.50h
Clove (*Syzgium aromaticum*)	5.00 ±0.00h	76.15 ± 1.14a
Thyme (*Thymus vulgaris*)	9.67 ±0.58g	53.98 ±1.40b
Mustard (*Brassica nigra*)	11.33 ±0.58f	46.02 ±1.40c
Black cumin (*Nigella sativa* L.)	14.00 ±0.00e	33.23 ±3.18ef
Garden cress (*Lepidium sativum*)	13.67 ±0.58e	34.82 ± 4.26de
Ethiopian mustard (*Brassica carrinata*)	16.00 ±1.00bc	23.62 ±7.11gh
Garlic (*Allium sativum*)	16.33 ±0.58bc	22.03 ±6.33h
Turmeric (*Curcuma longa*)	16.67 ±0.58b	20.59 ±1.91h
Coriander (*Coriandrum sativum*)	8.67 ±0.58g	58.66 ± 3.47b
Black pepper (*Piper nigrum* L.)	14.67 ±0.58de	30.13 ±1.63efg
Garden Rue (*Ruta chalepenis* L.)	12.33 ±0.58f	41.10 ±5.44cd
Moringa (*Moringa oleifera*)	16.33 ±0.58bc	22.18 ±1.96h
Rosemary (*Rosmarinus officinalis*)	15.33 ±0.58cd	26.80 ±6.11fgh
Control (Only *Xap*)	21.00 ±1.00a	

Means that do not share a common letter within a column are significantly different from each other at P≤0.05 according to DMRT.

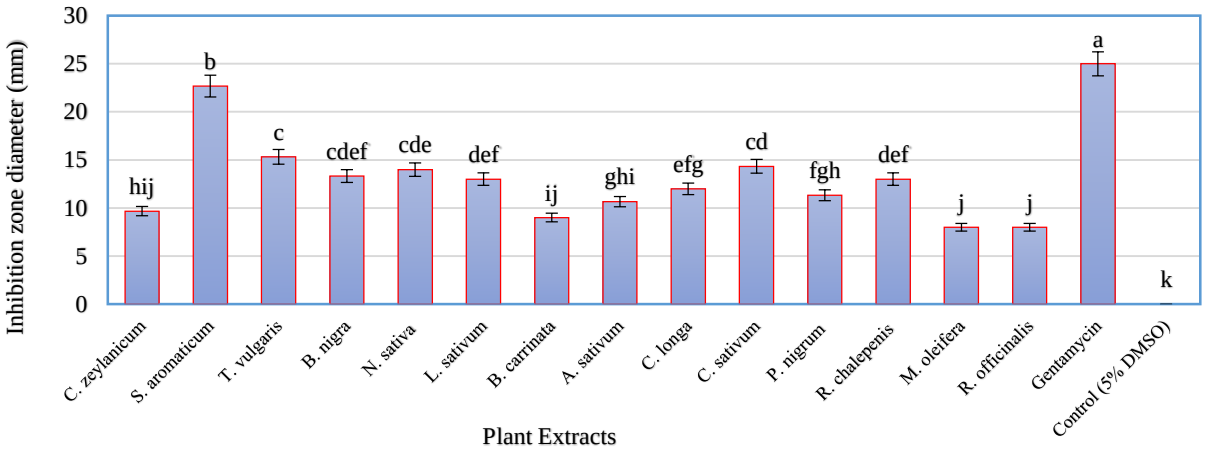

Figure 1. Antibacterial effects of 14 aromatic and medicinal plant extracts against Xap. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

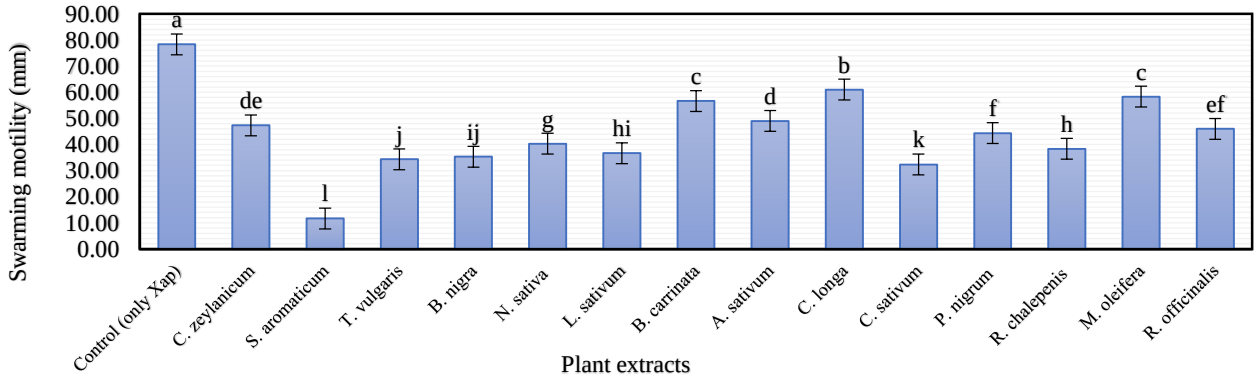

Figure 2. Swarming motility (mm) of Xap in the presence of aromatic and medicinal plant extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

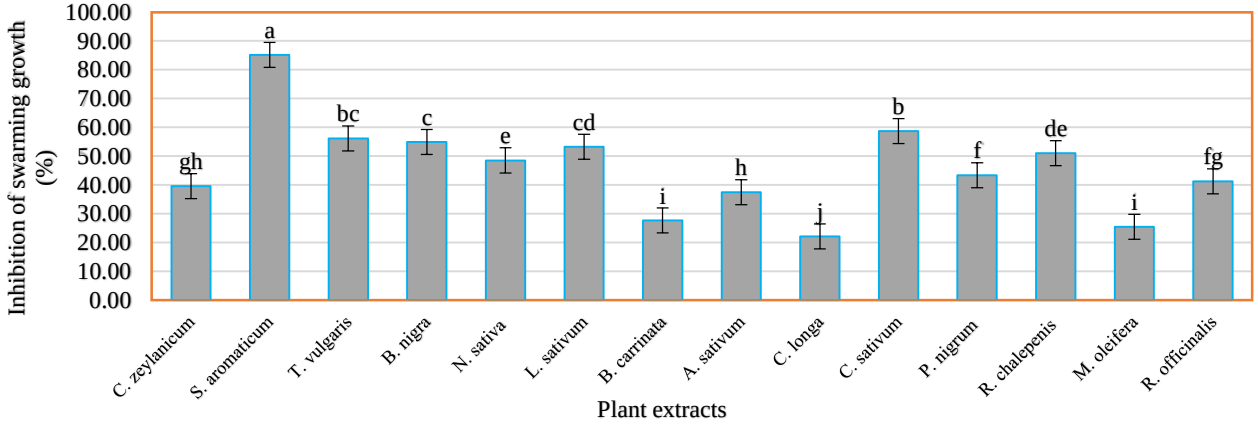

Figure 3. Percent inhibition of swarming motility of Xap in the presence of aromatic and medicinal plant extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

The *S. aromaticum* extract reduced the swarming motility by 85%, swimming motility by 89%, and twitching motility by 76% over the control treatment (Table 3-5 and Fig. 2-7). In addition to *S. aromaticum*, a satisfactory result in inhibition of swarming, swimming, and twitching motility were also recorded in *C. sativum*, *T. vulgaris*, *B. nigra*, *L. sativum*, and *R. chalepenis* over control treatment. *C. sativum*, *T. vulgaris*, *B. nigra*, *L. sativum*, and *R. chalepenis* extracts reduced swarming in Xap by 58.69, 56.16, 54.88, 53.20, and 51.04%, respectively. They also reduced the swimming motility by 59.10, 57.68, 54.33, 50.96, and 52.37%, respectively. These results indicated that crude extracts of *S. aromaticum*, *C. sativum*, *T. vulgaris*, *B. nigra*, *L. sativum*, and *R. chalepenis* had the greatest effect on swimming, swarming, and twitching motility of Xap. The least swimming, swarming, and twitching motility effect was observed by *B. carrinata*, *M. oleifera*, and *C. longa*. Their inhibition motility effect was less than by 40%. Even though they had less effect as compared to other plant extracts, they were significantly different by far from the control treatment.

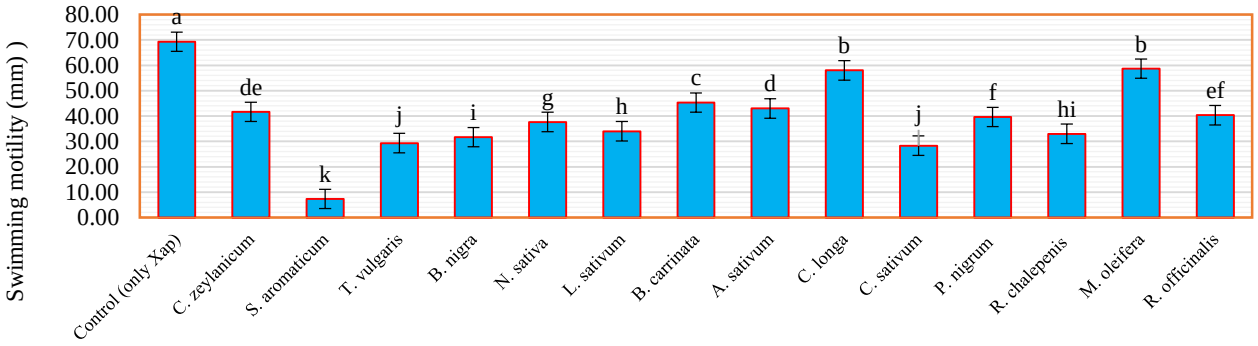

Figure 4. Swimming motility (mm) of Xap in the presence of some aromatic and medicinal plant crude extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

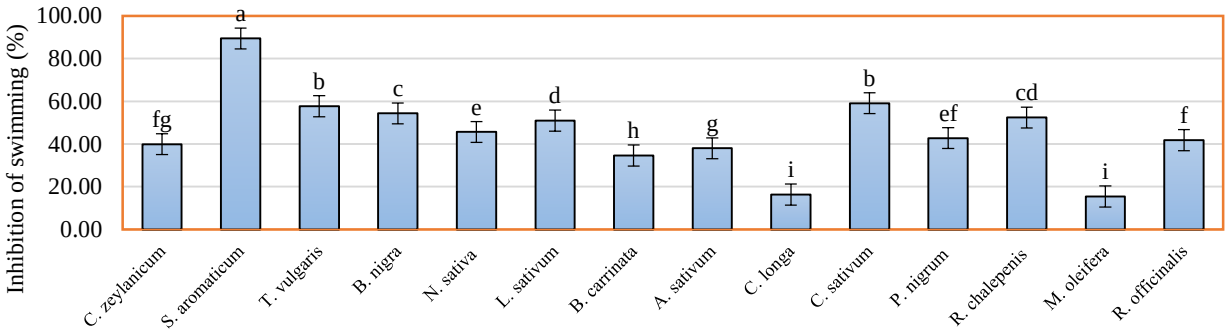

Figure 5. Percent inhibition of swimming motility of Xap in the presence of some aromatic and medicinal plant crude extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicatstandard errors of the mean.

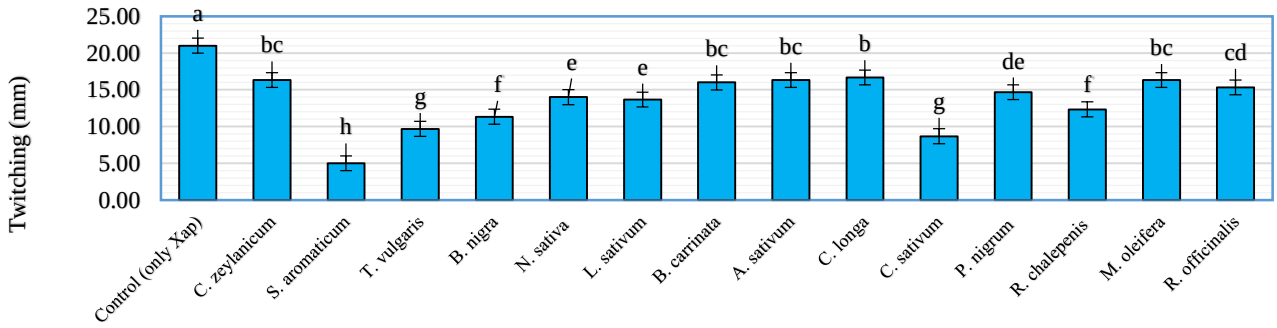

Figure 6. Twitching motility (mm) of Xap in the presence of aromatic and medicinal plant extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

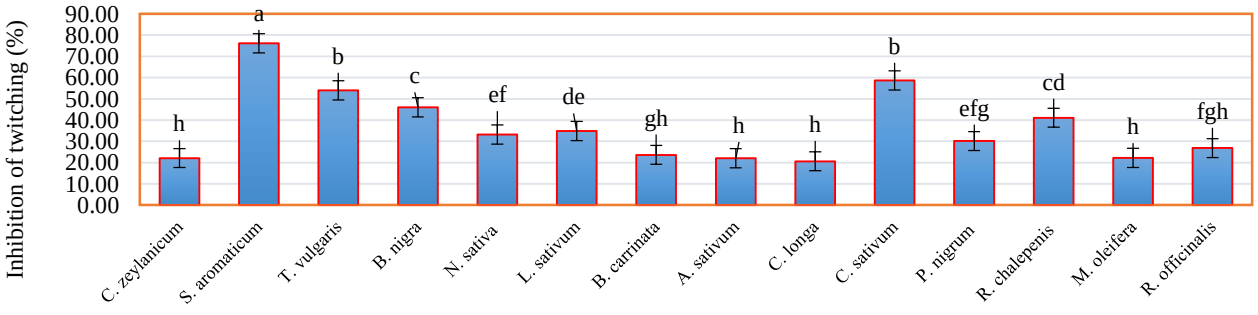

Figure 7. Percent inhibition of twitching motility of Xap in the presence of aromatic and medicinal plant extracts. Means that do not share a common letter in each bar are significantly different according to DMRT (P≤0.05) and vertical bars indicate standard errors of the mean.

Discussion

Nowadays, Xap on bean plants exhibits resistance to many synthetic chemical compounds and antibiotics. Due to this problem, scientists are trying to develop new compounds to treat pathogenic bacteria as a biological control mechanism. That's why the use of plant extracts as an antibacterial effect is very important for eco-friendly management of plant diseases by reducing the usage of chemical compounds. In addition, developing eco-friendly compounds which are inhibiting and disturb the communication of bacterial pathogens (quorum sensing) is one of the most important approaches. Several strategies were set to inhibit the bacterial QS system. Disturbing and inhibiting QS signaling molecules and inhibiting bacterial diffusion (motility) are among the best strategies (Bouyahya et al., 2017).

The antibacterial bacterial activity of *S. aromaticum* extract in this study strongly agreed with the results of Benchouikh et al. (2016) who found that *S. aromaticum* had a strong antibacterial effect against the tested Xap isolates. Likewise, in Indian, Chudasama and Thaker (2012) were evaluated the antibacterial effect of many aromatic plant oil extracts against *Xanthomonas campestris* pv. *citri* indicated that *S. aromaticum* and *T. vulgaris* had strong zones of inhibition 24.33 and 23.66 mm, respectively. Hosseinzadeh et al. (2013) were reported *T.vulgaris* oil exhibited higher activity against *R. solanacearum*. Next *S. aromaticum* and *T. vulgaris* plant extracts, *C. sativum* was showed a potential antibacterial effect against Xap. A research result in Italy indicated C. *sativum* oil strongly inhibited the growth of *E. coli* and *B. megaterium* (Lo Cantore et al., 2004). In addition, the authors also reported *C. sativum*inhibited the growth of different strains of both gram-negative and gram-positive plant pathogenic bacteria (*Pseudomonas*, *Erwinia*, *Xanthomonas*, *Agrobacterium*, *Clavibacter*, *Curtobacterium*, and *Rhodococcus*). Similarly, Ibrahim and Abu-Salem (2014) reported, the methanol extracts of *S. aromaticum* showed high potential antibacterial activity against *Bacillus cereus*, *Salmonella typhi*, and *Staphylococcus aureus*. Aromatic and medicinal plants have different phenolic compounds that inhibit the activity of both gram-positive and negative bacteria. Sharma et al. (2014) and Benchouikh et al. (2016) reported that phenolic acids as a secondary plant metabolite such as eugenol, which is found in *C. zeylanicum* and *S. aromaticum*, have an antibacterial effect. Likewise, Soltani and Aliabadi (2013) reported the antibacterial activity of plant extracts is related to a number of flavonoids and phenolic compounds which are in pure form. Kasa and Woldeab (2015) were also evaluated the crude extracts of different plants against *Xanthomonas campestris* pv. *musacearum* in Ethiopia and identified a promising result for this pathogen.

Plant extracts have the ability to inhibit the expression of specific induced gene (s) during bacterial communication (El-Hamid, 2016). They are very important tools for the management and control of bacterial pathogenesis through modulation of bacterial virulence genes. In this study, the effect of crude plant extracts extracted from the different parts of aromatic and medicinal plants on swarming, swimming, and twitching motility of Xap was investigated. Among the tested plants *S. aromaticum* was the most effective in all assays (swarming, swimming, and twitching) over the control treatment. Small swimming, swarming, and twitching diameters were observed in *S. aromaticum* extract treatment which was in contrast with the control sets where the large diffused colony was observed on the swarm, swim, and twitch plates. Our results indicate that some aromatic and medicinal plant extracts were limit the migration of Xap bacteria on plates. Similar results investigated by You et al. (2007) and Koh and Tham (2011) the bud extract of *S. aromaticum* inhibit the QS-mediated virulence factors (swimming, twitching, and swarming) of *Pseudomonas aeruginosa*. Likewise, among the 21 essential oils screened by Khan et al. (2009), a significant QS inhibition effect was observed in *S. aromaticum* oil against *C. violaceum* strains. These authors reported a 78% reduction in swarming motility by *S. aromaticum*on *P. aeruginosa*. In addition, a study by Ganesh and Rai (2015) indicated essential oils of *S. aromaticum, R. officinalis, C. verum,* and *C. longa* has shown anti-QS activity against *P. aeruginosa.* They also reported the essential oils of *C. verum* and *S. aromaticum* decreased the swarming motility of the tested bacterial pathogens. The QS inhibition effect of *C. zeylanicum* was relatively less intense against the *C. violaceum* strains (Khan et al., 2009). According to Pfeilmeier et al. (2016), bacterial pathogen must migrate from the epiphytic surface to the inside tissue of the infecting plant using motility and chemotaxis machinery to get successful infection development. *C. sativum* and *T. vulgaris* had also a strong effect on the motility assay of Xap isolate. This indicates that both aromatic and medicinal plant extracts constitute a promising ingredient for the management and control of bacterial disease through the modulation of bacterial virulence factors (El-Hamid, 2016). A research result by Singh et al. (2017a) demonstrated *T. vulgaris*oil had a considerable reduction effect in swarming and swimming motility (1.24 and 1.19 fold, respectively) as compared to the control treatment. According to Al-Haidari et al. (2016), the anti-quorum sensing effect of alcohol extracts of medicinal plants was evaluated against *C. violaceum* bacteriaand the result revealed that *C. sativum and A. cepa* had a significant effect. They also indicated these plant extracts manifested a distinct effect on motility (swarming and twitching) of *P. aeruginosa.*

The production of surfactant molecules which mainly play an important role in facilitating the swarming motility of bacteria are triggered by acyl-homoserine lactones (AHLs) (Eberl et al., 1996). Any natural plant compound that inhibits the swarming, swimming, and twitching motility is more likely to hinder the production of quorum sensing molecules.

Conclusion

The results from our antibacterial study indicate that the use of different aromatic and medicinal plant extracts especially *S. aromaticum, T. vulgaris, C. sativum, N. sativa, B. nigra, L. sativum* and *R. chalepenis* were showed a potential antibacterial effect on Xap isolate under in-vitro

conditions and may use as a natural bactericide (biological control) for the management of bean bacterial disease caused by Xap under field conditions and for seed treatment in organic agriculture.

Generally, in our study, the plant extracts from *S. aromaticum*, *C. sativum*, *T. vulgaris*, *B. nigra*, *L. sativum*, and *R. chalepenis* were more effective and superior to the remaining tested plant extracts indicated antibacterial activities and swimming, swarming, and twitching motility inhibition and recommended that the importance of aromatic and medicinal plant extracts as a rich source of compounds able to inhibit QS mediated virulence factors. They could manage CBB disease development and hinder its dissemination. Therefore, further investigation on the nature of these anti-QS plant extract compounds on this particular bacterium and their mode of action is still required.

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Conflicts of interest

The authors declare that they have no competing interests.

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