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Antibacterial Activity and Phytochemical Screening of *Tagetus erecta* L. and *Pelargonium graveolens* against *Xanthomonas axonopodis* pv. *punicae*

G.L. ARUNA

Department of Microbiology, Maharani's Science College for Women, Mysore, Karnataka, India.

Abstract

The antibacterial activity of selected medicinal plants namely *Tagetus erecta* L. and *Pelargonium graveolens* extracts was studied against *Xanthomonas axonopodis* pv. *punicae*, the causative agent of bacterial blight of *Punica granatum.* The plants are selected based on their traditional use as medicinal plants.The water, ethanol and methanol extracts of *Taget erecta* L. and *Pelargonium graveolens* were used for antibacterial activity assay by Kirby Bauer disc diffusion method. Among these extracts, only methanol extract of *Taget erecta* L. has showed substantial antibacterial activity against *Xanthomonas axonopodis* pv. *punicae* and all other extracts showed less antibacterial activity. The phytochemical constituents such as alkaloids, flavonoids, tannins, proteins, resins, proteins, saponins, terpenoids, steroids, and glycosides were found to be present in selected plant extracts as per preliminary phytochemical investigation. The Minimum inhibitory concentration and Minimum bacteriocidal concentration of *Tagetus erecta* L. methanol extract was found to be 25mg/ml and 50mg/ml respectively. Our study revealed the possible application of *Tagetus erecta* L. methanol extract in the management of Bacterial blight of *Punica* granatum.

Article History

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Keywords

Phytochemical Constituents; Tannins; *Xanthomonas axonopodis*; Anti bacterial Activity; *Tagetus erecta* L; Minimum Bacteriocidal Concentration.

Introduction

The pomegranate tree is indigenous to the Mediterranean region of Asia, Africa, and Europe and can be found growing from Iran to the Himalayas in Northern India. It is considered as the "Fruit of Paradise" because it is most economically benificial and it shows various medicinal properties.[1] Maharashtra, Karnataka, Andhra Pradesh, Rajasthan, Gujarat, and Tamil Nadu are among the Indian states where it is cultivated.[2] In field this plant is susceptible to various diseases namely Anthracnose caused by *Colletotrichum* sp., Fruit blight caused by *Alternaria* sp., Leaf spot and fruit spot caused by *Cercospora* sp., Scab caused by *Sphaceloma* sp., wilt caused by *Ceratocystis fimbriata* and *Fusarium oxysporum* and bacterial

CONTACT G.L. Aruna microarunagl@gmail.com Department of Microbiology, Maharani's Science College for Women, Mysore, Karnataka, India.

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blight caused by *Xanthomonas axonopodis* pv. *punicae.* Among these blight disease of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* is more severe.[3] The bacterial blight disease of pomegranate was first noticed in India, where the lack of efficient management techniques poses a serious danger to pomegranate farming. In India it is resulting in severe production losses of about 60–80% depending on disease severalty.[1] In the year 2000, about 90% of Karnataka's pomegranate cultivated land was damaged in the districts of Bagalkot, Belgaum, Bellary, Bijapur, Chitradurga, Gulburga, Koppal, Raichur, and Tumkur.[4]

Xanthomonas axonopodis pv. *punicae* is non spore forming Gram negative rod shaped bacterium with single polar flagellum. It forms circular, smooth, glistening, light yellow, mucoid, convex with entire margins colonies on nutrient glucose medium.[5] It cause bacterial blight in pomegranate and it shows the initial symptoms that is appearance of water-soaked irregular to round, tiny black dots on leaves eventually develop a necrotic centre and turn to a dark brown colour. Spots can grow into a mass and create a large patch in severe cases, which may cause diseased leaves to fall off. On stem nodes, dark spot symptoms appear and begin cracking, easily break off the branches and cracking of fruits.[6] Currently, streptomycin and copper oxy-chloride are being used to control bacterial blight disease.[5] These chemicals are not economical and eco-friendly. Hence medicinal plants may be explored as one of the eco-friendly strategy for the control of bacterial blight disease of pomegranate. Some of the medicinal plants showed antibacterial activity against some plant pathogens. Bharadwaj and Laura in 2009 have reported that *Xanthomonas campestris* pv. *campestris* was inhibited by *Acacia arabicae, Acacia catechu, Aegle marmelos*, *Asparagus racemosus*, *Achryanthus asper*, *Azadirachta indica, Callistemon lanceolatus* and *Acacia fernesiana*.[7] In this context an attempt is made to study the antibacterial property of commonly available two selected medicinal plants namely *Tagetus erecta* L. and *Pelargonium graveolens* against *Xanthomonas axonopodis* pv. *punicae. Tagetus erecta* L. popularly known as Marigold. Singh *et al,* reported the wound healing, antibacterial, antimicrobial, antiepileptic, insecticidal, larvicidal, hepatoprotective, antipyretic and antifungal properties of *Tagetus erecta* L.[8] *Pelargonium graveolens* commonly known as rose geranium. Hamidpour *et al.*, reported the antifungal, antioxidant and hypoglycemic activities of *P. graveolens*

Fig. 1: Symptoms of Bacterial blight of pomegranate on (a) leaves, (b) stem and (c) fruit part

Materials and Methods

Collection of Diseased *Punica granatum* Plant

Punica granatum (pomegranate) diseased plant was collected at the village Heggere, Challakere (Tq), Chitradurga (Dist.), Karnataka in January, 2022 and diseased plant leaves, stem and fruit parts were plucked from plant.

Koch's Postulates

The causal relationship between *Xanthomonas axonopodis* pv. *punicae* and *Punica granatum* was proved by Koch's postulates.

Collection and Identification of Medicinal Plant Materials

The medicinal plants were collected from different parts of Chitradurga district. These plants include *Tagetus erecta* L. commonly known as Marigold and *Pelargonium graveolens* commonly known as lemon geranium in Doddasiddavanahally, Chitradurga, Karnataka, India. They are identified and authenticated by Dr. Lingannaiah and Prof. R. K. Rangaswamy, faculty of the Department of Botany, Government science college, Chitradurga using the gamble flora of Madras presidency.

Preparation of Plant Extracts

Fresh healthy leaves and flowers were collected from selected plants. They were washed individually once with under running tap water and with sterile water to get rid of dirt and other debris. The leaves and flower petals were shade dried at room temperature for 3-5 days in the laboratory. A fine powder of dried selected plant materials was made using pestle and mortar. The powdered plant material was stored at room temperature in air sealed polythene bags till the extract was prepared.[10]

About 20 gm of selected plant material powders were immersed separately in solvents such as 100 ml of distilled water, ethyl alcohol and methyl alcohol for 24 hrs. After incubation, each soaked plant material was mixed vigorously and filtered through Whatmann filter paper no.1 and the filtrate was subjected to drying by rota evaporator. Then the plant leaf and flower extracts were used for *in vitro* antibacterial activity assay, phytochemical screening, minimum inhibitory concentration and minimum bacteriocidal concentrtaion studies.[7]

Preliminary Phytochemical Screening

The preliminary phytochemical studies were carried out by the accepted methods described by Kokate *et al.*[11] with some changes. The leaf and flower extracts of *Tagetus erecta* L. and *Pelargonium graveolens* were assayed for the detection of phytochemical constituents such as flavonoids, resins, proteins, terpenoids, glycosides etc.

Anti Bacterial Assay

Isolation of Bacterial Culture

The *Xanthomonas axonopodis* pv. *punicae* was isolated from diseased pomegranate plant material using nutrient agar. Diseased plant material is placed on sterilized nutrient agar medium plates and incubated at 37° C for one day. The isolated bacterium from *Punica granatum* is identified as *Xanthomonas axonopodis* pv. *punicae* based on Gram staining and biochemical tests.[12]

Preparation of Bacterial Inoculum

The *Xanthomonas axonopodis* pv. *punicae* isolated from *punica granatum* was identified as *Xanthomonas axonopodis* pv. *punicae* based on Gram staining and biochemical tests was cultured in nutrient broth and incubated for overnight at 37° C and centrifuged at 1000 rpm for 5 min. The bacterial pellet was collected and suspension was prepared using double distilled sterile water.

Preparation of Plant Extracts Discs

The sterilized Whatmann paper No.1 was cut into circular discs of 6 mm diameter. The discs were sterilized by autoclaving. The discs were soaked in plant extracts, dried and preserved at 4° C until further use.

Antibacterial Activity Test

The anti-bacterial activity was determined by Kirby-Bauer method. Nutrient agar medium plates were labeled with the name of plant extract to be placed and the bacterial culture to be inoculated. A volume of 0.1ml of *Xanthomonas axonopodis* pv. *punicae* suspension was placed on nutrient agar medium plates using sterile pipette and the inoculum was uniformly spread over agar surface using L-shaped glass rod. The presoaked and air dried plant extract discs were placed on their respective Petri plates. Standard antibacterial agent discs (Streptomycin

and Copper oxychloride) which are used to control bacterial blight of pomegranate were placed in other bacterial plate. All the plates were incubated at 37°C for 24 hrs and the plates were visualized for the presence of zone of inhibition. The diameter of zone of inhibition was documented. The antibacterial activity test was performed in triplicates.

Minimum Inhibitory Concentration (MIC)

The lowest concentration of the plant extract which shows no growth is visualized in the test tube is known as Minimum inhibitory concentration (bacteriostatic concentration). The MIC and MBC of selected plant extracts was detected by method described by Usman *et al.*, (2007). The broth double serial dilution method was used to determine the MIC and MBC.[13] The *Tagetus erecta* L. methanol extract was serially double diluted from 100 mg/ml to 6.25 mg/ml in nutrient broth medium.

A volume of 0.5 ml suspension of the test organism was inoculated to all the test tubes containing nutrient broth and plant extract in different concentration. After 24 hours of incubation at 37^0C, the test tubes were looked for growth of bacteria. The calorimetric method was used to determine the growth of bacteria. The test tube containing plant extract at lowest concentration that inhibited the growth of the test organism completely (no turbidity) was the minimum inhibitory concentration (MIC) of the selected plant extracts.

Minimum Bactericidal Concentration (MBC)

The lowest concentration of plant extract where bacterial growth is not observed is known as MBC. This was determined by sub culturing the sample from broth dilutions of MIC tubes to nutrient agar medium. In this method, the sample from the MIC test tubes showing no turbidity was inoculated on sterile nutrient agar plates using a L-shaped glass rod and incubated at 37^0C for 24 hours. The least concentration of the plant extract which did not showed bacterial growth on the inoculated nutrient agar plate was the MBC.

Results and Discussion

Phytochemical Analysis: Latitude: 13.991, Longitude: 75.49815)

The preliminary phytochemical analysis of aqueous, ethanol and methanol extracts of *Taget erecta* L. and *Pelargonium graveolens* prepared in the year 2022 and stored at refrigerator until processed. It revealed the presence of alkaloids, flavonoids, tannins, resins, proteins, saponins, terpenoids, steroids and glycosides as shown table 1. The aqueous extracts of both *Taget erecta* L. and *Pelargonium graveolens* showed the presence of all tested phytochemical constituents.

Table 1: Phytochemical analysis of *Tagetus erecta* L. and *Pelargonium graveolens* extracts

Sl. No	Active compound	Type of extracts					
		***Tagetus erecta* L. extracts**			***Pelargonium graveolens* extracts**		
		Aqueous	**Ethanol**	**Methanol**	**Aqueous**	**Ethanol**	**Methanol**
1	Alkaloids	+	+	+	+	+	+
2	Flavanoids	+	+	+	+	+	+
3	Tannins	+	-	-	+	-	-
4	Saponins	+	-	+	+	-	-
5	Resins	+	+	+	+	+	+
6	Cardiac glycosides	+	-	+	+	+	+
7	Steroids	+	-	-	+	+	+
8	Terpinoids	+	+	+	+	-	+

Note: “+”=Present, "-" =Absent

The ethanol extracts of *Tagetus erecta* L. has shown alkaloids, flavanoids, resins and terpenoids and the ethanol extracts of *Pelargonium graveolens* has shown alkaloids, flavanoids, resins, cardiacglycosides and steroids. The methanol extracts of *Tagetus erecta* L. has revealed the presence of alkaloids, flavanoids, saponins, resins, cardiacglycosides and terpenoids. The methanol extracts of *Pelrgonium graveolens* has shown the presence of alkaloids, flavanoids, resins, cardiacglycosides, steroids and terpenoids

Antibacterial Assay

As per antibacterial assay, among three different extracts of *Tagetus erecta* L. only methanol extract showed maximum inhibition zone against *Xanthomonas axonopodis* pv. *punicae.* All other extracts of *Tagetus erecta* and extracts of *Pelargonium graveolens* have showed less inhibition activity as shown in figure 1 and table 2. This might be due to less affinity of phytochemical active ingradients of *Tagetus erecta* and *Pelargonium graveolens* to water and ethanol. Among three extracts of two different selected plants only methanol extract of *Tagetus erecta* L. more effective inhibitor of *Xanthomonas axonopodis* pv. *punicae.*

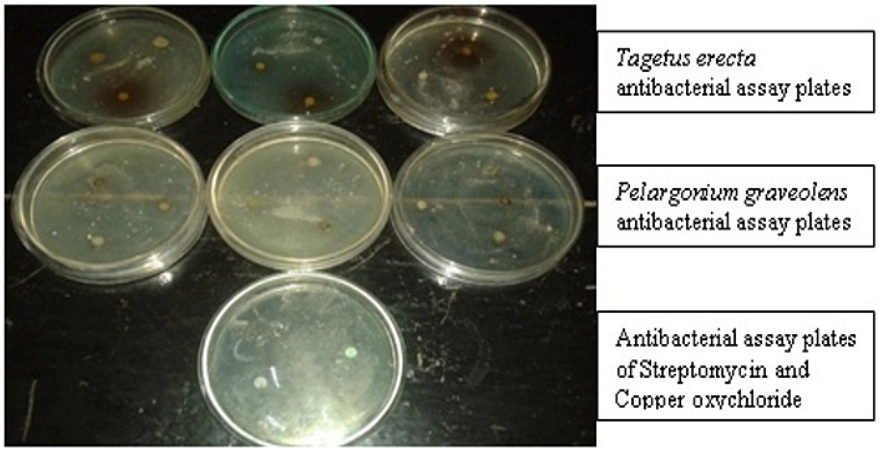

Fig. 2: Antibacterial activity of selected medicinal plant extracts against *Xanthomonas axonopodis* pv. *punicae* by Kirby-Bauer disc diffusion method.

Table 2: Antibacterial activity of selected plants extracts against *Xanthomonas axonopodis* pv. *punicae*

Plant used	Diameter of zone of inhibition (in mm)		
	Aqueous Extract	**Ethanol Extract**	**Methanol Extract**
Tagetus erecta	3 mm	0	19 mm
Pelargonium graveolens	1 mm	1 mm	2 mm
Streptomycin sulphate + tetra cycline hydrochloride		39 mm	
Copper oxy chloride		12 mm	

The previous workers reported the anti bacterial activity of different medicinal plant extracts against different species of *Xanthomonas.* Babu *et al.*, in 2007 reported that *Origanum vulgare* and *Althea officinalis* are potential candidate plants for the management of phytopathogenic *Xanthomonas*

sp.[14] Bharadwaj *et al.*, in 2011 has reported that the leaves, stem, flowers and fruits of *Azadirchata indica* plant extracts showed inhibitory effects on growth of *Xanthomonas campestris.*[7]

Minimum Inhibitory and Bactericidal Concentration

As only methanol extract of *Tagetus erecta* L. showed significantly high inhibitory activity against *Xanthomonas* pv. *punicae*, the minimum inhibitory and bactericidal concentration studies were done only for this extract. The MIC and MBC values are shown in table 3. Previous studies reported the MIC and MBC assays of different plant extracts on different bacteria. Kang *et al.*, in 2011 have also conducted the MIC and MBC of methanol extract of medicinal plants against Gram-positive (5 strains) and Gram-negative bacteria which showed the highest zone of inhibition in methanol extract.[13] This study demonstrates the presence of phytochemical constituents is responsible for this inhibitory activity. Application of medicinal plants as biocontrol agents is promising strategy for efficient control of bacterial blight disease.

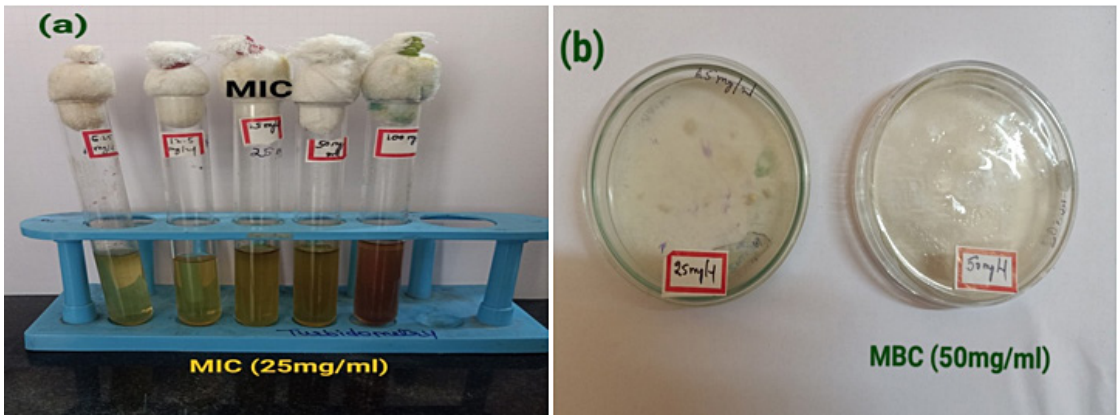

Fig. 3: (a) MIC and (b) MBC of *Tagetus erecta* L methanol extracts against *Xanthomonas axonopodis* pv. *punicae*

Table 3: MIC and MBC of the *Tagetus erecta* L. methanol extract

Sl. No.	Bacteria	MIC (mg/ml)	MBC (mg/ml)
1.	*Xanthomonas axonopodis* pv. *punicae*	25mg/ml	50mg/ml

Conclusion

There is an increasing interest in the scientific research for medicinal plants because of their potential applications in medicines and plant disease control measures. Considering the wide variety of plants, it is anticipated that scientific testing of plant extracts for antibacterial activity will lead to the discovery of new antibacterial compounds. The current study findings provide a scientific basis for traditional application of *Tagetus erecta* L in controlling the diseases. However, further work on these medicinal plants is required to determine their active phytochemical constituent, which is inhibitory to *Xanthomonas axonopodis* pv. *punicae.* The antibacterial activity could be increased if active components are obtained in purified form.

There is an increasing interest in the scientific research for medicinal plants because of their potential applications in medicines and plant disease control measures. An attempt was made to study the antibacterial property of two selected medicinal plants namely *Tagetus erecta* L. and *Pelargonium graveolens* against *Xanthomonas axonopodis* pv. *punicae.* The current study findings provide a scientific basis for traditional application

of *Tagetus erecta* L extract in controlling the pomegranate diseases. However, further work on this medicinal plant is required to determine their active phytochemical constituent, which is inhibitory to *Xanthomonas axonopodis* pv. *punicae.* The antibacterial activity could be increased if active components are obtained in purified form. Further work aimed at HPLC analysis of *Tagetus erecta* methanol extract.

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Conflict of Interest
The authors declare that they have no Conflict of Interest.

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