



## ***In vitro* antibacterial activity of *Pituranthos scoparius* from Algeria**

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### ABSTRACT

Traditional medicine has a key role in health care worldwide; the search for antimicrobial agents from plants has been a growing interest in the last few decades. In the present study, antibacterial properties of *Pituranthos scoparius* were explored. Aerial and root parts of the plant were extracted with a series of solvent of varying polarity including water, methanol, acetone and chloroform. The antibacterial activity of extracts was assessed by agar disc diffusion method and broth microdilution method against 14 Gram positive and Gram negative pathogenic bacteria. The extracts from the aerial parts have shown a better antibacterial activity than the root extracts. The acetone aerial part extract showed the highest activity (about 22 mm inhibition zone) against *Proteus mirabilis*, and a MIC of 1.04 mg/mL against *Salmonella* Typhimurium, followed by the methanolic aerial part extract (about 15mm inhibition zone), with a MIC of 1.56 against *Enterococcus faecalis*. The values of MIC obtained show that the extracts have weak activities since all MIC values are greater than 1 mg/ml, comparing with the references antibiotics. The findings of the study indicate that *Pituranthos scoparius* could be a new source of antibacterial natural drugs. *In vivo* studies remain necessary to ensure the antibacterial efficacy of the plant.

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**Keywords:** *Pituranthos scoparius*, medicinal plants, extracts, disk diffusion method, minimum inhibitory concentration.

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### INTRODUCTION

Antibiotics provide the main basis for the therapy of microbial (bacterial and fungal) infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents, there has been a belief, in the medical community, that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major reason for the emergence and dissemination of multidrug resistant bacteria (Mohammed et al., 2012). Nowadays, antimicrobial resistance is a growing problem that complicates the treatment of important nosocomial and community-acquired

infections (Emmanuel et al., 2012). In the last few decades, this situation has forced scientists to search for new and effective antimicrobial agents from plants (Jacquelyn, 2002; Mukhrizah et al., 2011).

Furthermore, nature is an ancient source of medicinal agents and an impressive number of modern drugs have been isolated from natural sources; many of them based on their use in traditional medicine. A special feature of higher plants is their capacity to produce a large number of organic chemicals of high structural diversity, the so-called secondary metabolites, and have an ecological

importance (Jahir and Saurabh, 2011). These chemical compounds are found in various parts of medicinal plants, like the stems, roots, leaves, barks, flowers, fruits and seeds (Cutter, 2000). The most important of these medicinal compounds are alkaloids, tannins, flavonoids and phenolic compounds (Amal et al., 2009), which are synthesized and deposited in specific or all parts of the plant. Over the past decade, there has been an explosion of interest to natural products drug discovery, and in the development of new antimicrobial agents, as they offer a virtually unlimited source of unique molecules (Melissa and Larry, 2005). Nowadays, several studies focus on searching for antibacterial properties of plant extracts and phytochemicals (Ngwendson et al., 2003; Recio and Rios, 1989).

Algeria, a country in Northern Africa, is characterized by high floral diversity and significant rates of endemism. Many plants have been used since ancient times, for their perfume and flavor as well as in food preservation and medical applications.

In traditional medicine, stems and leaves of *Pituranthos scoparius* have been used in the treatment of measles, rheumatism, asthma, jaundice, digestive difficulties, urinary infections, diabetes, hepatitis and postpartum care: spasms and pains. They are also still used against snake-bites and scorpion-bites. *P. scoparius* leaves, in powder form, are recommended for local applications, as a poultice (Hammiche and Maiza, 2006; Boukef, 1986). This medicinal plant was chosen in the present study, on the basis of its ethnobotanical benefits.

Previous studies showed that the essential oils of *P. scoparius* are rich in  $\alpha$ -pinene,  $\beta$ -pinene, limonene, myristicin, dillapiolene and germacrene-D (Hammiche and Maiza, 2006; Vérite et al., 2004; Smaili et al., 2011; Gourine et al., 2011; Takia et al., 2013).

The aim of the present study was to investigate the antibacterial activity of extracts of *Pituranthos scoparius*, locally called "guezzah". It is from the Apiaceae, an aphyllous (or almost) perennial plant; the

upper leaves are reduced to their sheath, ending in a point. The stems are erect, 40-80 cm. high, and form dense clumps that send out laterally short stiff branches. The flowers, with an often short peduncle, white petals and narrow veins, are bunched in lateral umbels that are fairly spread out, with 4-8 spindly spokes. The fruit is a 1.3 mm long globular mericarp. Flowering occurs from February to October (Quézel and Santa, 1962). It is an endemic species of North Africa region, widespread in the central Sahara (Ozenda, 1983).

## MATERIELS AND METHODS

### Plant material

*Pituranthos scoparius* (Coss. & Dur.) Benth. & Hook, was collected from the region of Bechar, in southwestern Algeria, in September 2012, by the end of flowering season. The taxonomic identification of the plants was done in the Botany Department, at Tlemcen University, Algeria. The samples were air-dried in the shade in order to preserve their properties.

### Extract preparation

After drying the plant material: aerial part (consisting of the stem and flower) and root part was powdered. Crude extracts were obtained by maceration, for 24 hours at room temperature, with a series of solvents of varying polarities (distilled water, methanol, acetone and chloroform), at a ratio of 1/10 of dry material to solvent in each case.

### Preliminary phytochemical screening

A phytochemical analysis of *P. scoparius* was conducted for the detection of secondary metabolites such as tannins, alkaloids, flavanoids, terpenoids, sterols and/or terpenes, quinones, coumarins, anthraquinones and saponins; these were evaluated by standard qualitative methods of Trease and Evans (1989).

### Evaluation of the antibacterial activity

In this study, we used a panel of fourteen different American Type Culture

Collection (ATCC) reference pathogenic bacteria obtained from our laboratory: “Antibiotiques Antifongiques: Physico-Chimie, Synthèse et Activité Biologique”, at the Department of Biology, Faculty of Sciences, Tlemcen University, Algeria.

The following bacteria species were used; the Gram negative species: *Escherichia coli* ATCC 25933, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Citrobacter freundii* ATCC 8090, *Proteus mirabilis* ATCC 35659, *Salmonella* Typhimurium ATCC 13311, *Enterobacter cloacae* ATCC 13047; and Gram positive species: *Staphylococcus aureus* ATCC 25923, methicillin-resistant *Staphylococcus aureus* MRSA ATCC 43300, *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 49452, *Listeria monocytogenes* ATCC 15313.

For this purpose, two methods, recommended by the Clinical and Laboratory Standards Institute (CLSI) were used; the disk diffusion method and the microdilution method.

#### **The disk diffusion method**

The agar disk diffusion method was performed to assess the antibacterial potential of extracts, based solely on the presence or absence of a zone of inhibition (CLSI, 2010a). Each extract was dissolved in dimethyl sulfoxide (DMSO) to get 200 mg/ml final concentration. Gentamicin (10 µg) and cephalexin (100 µg) served as the positive antibacterial controls. A pure DMSO as used as the negative control.

The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standards, which resulted in a suspension containing approximately  $10^8$  CFU/ml; a spectrophotometer was used ( $DO = 0.08 - 0.13/\lambda = 625$  nm).

The plates were inoculated with the suspension adjusted on Mueller-Hinton agar by cotton swab. After drying, the sterile filter paper disk (6 mm diameter) was then impregnated with 10 µl of each extract. The

plates were incubated at 37 °C, for 18-20 hours. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the disks. All the tests were repeated three times to minimize test errors.

#### **Determination of minimum inhibitory concentration**

The minimum inhibitory concentration (MIC) of the extracts was determined by the microdilution method (CLSI, 2010b).

First, the inoculums were prepared and all the bacterial species were adjusted to 0.5 McFarland standard turbidity. Next, serial doubling dilutions in Muller Hinton broth were employed to obtain the final concentration in well of  $5 \cdot 10^4$  CFU/ml.

In this test, sterile 96 well microplates were used. The extracts were then prepared and transferred to each microplate well, after having a twofold serial dilution in order to make a concentration range from 50 to 0.09 mg/ml.

The first well was used as a negative control; it was inoculated with the broth solely to check the sterility of the media. However, the last well was used as a positive control and was inoculated with the inoculum suspension. The microplates were incubated at  $35 \pm 2$  °C, for 16 to 20 hours, in an ambient air incubator. The MIC was the lowest concentration of the extracts that completely inhibited the growth of the microorganisms in the microdilution wells, as detected by the unaided eye.

#### **Determination of minimum bactericidal concentration**

To determine the minimum bactericidal concentration (MBC), the broth was taken from the wells which did not exhibit any visible growth in the MIC assay, then was cultured on freshly prepared sterile Nutrient agar and incubated at 37 °C for 18 - 24 hours. After incubation, the highest dilution (least concentration) that inhibited colony formation on a solid medium was considered as the MBC.

## RESULTS

### Preliminary phytochemical screening

The results of the phytochemical analysis of *P. scoparius* are given in (Table 1). The secondary metabolites commonly present were: flavanoids, terpenoids, tannins, steroids, quinons and coumarins; however, saponins and anthraquinones were not found in the extracts of all aerial parts and roots. Alkaloids were detected mainly in the chloroformic extracts.

### The disk diffusion method

The results of *in vitro* testing of the antibacterial activity of the different solvent extracts of *P. scoparius* are presented in the Tables 2 and 3. The qualitative test was to determine the presence or absence of inhibition zones around the disks.

The results obtained proved the existence of a modest to good activity against most of Gram positive bacteria and a certain number of Gram negative bacteria for aqueous-acetone, aqueous-methanol and chloroformic extracts. No activity was recorded for aqueous extracts against all pathogenic species. Among the plant extracts, acetone showed greater antibacterial activity (the higher inhibition zone) than methanol and chloroform extracts, but the methanol extract was more active against most strains. Extracts of the aerial parts showed maximum antibacterial activity compared to root extracts. The acetone extract from the aerial part showed highest activity, about 22.33 mm inhibition zone against *Proteus mirabilis* ATCC 35659 at 200 mg/ml, followed by the methanolic extract from the aerial part, about 15 mm against *Bacillus subtilis* ATCC 6633.

The lowest antibacterial activity was observed in the root part.

### The microdilution method

The results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts obtained from *P. scoparius* aerial part, using different solvents, against the tested organisms are shown in Table 4.

The minimum inhibitory concentrations of the extracts, which range from 1.04 mg/ml to 50 mg/ml. The minimum bactericidal concentrations of some of the most active extracts, which range from 3.12 mg/ml to 50 mg/ml, are also shown in Table 4.

The acetone extract of the aerial part was the most effective (with a MIC of 1.04 mg/ml and a MBC of 1.56 mg/ml) against *Salmonella Typhimurium* ATCC 13311. It was followed by the activities of the methanol extract (with MIC equal to 1.56 mg/ml and MBC to 3.12 mg/ml) against *Enterococcus faecalis* ATCC 25212 and those of chloroform extract whose MIC is 3.12 mg/ml and MBC of 3.12 mg/ml against *Staphylococcus aureus* ATCC 25923.

As for the root extracts, the results are shown in Table 5. The methanol extract was found to be the most effective (with MIC equal to 6.25 mg/ml and MBC to 6.25 mg/ml) against *Acinetobacter baumannii* ATCC 19606, followed by the chloroform and acetone extracts. Commercial gentamicin and cephotaxime showed higher antibacterial potency compared to the extracts tested (Table 6).

**Table 1:** Phytochemical screening of different extracts of aerial part and root of *P.scoparius*.

	Extracts	Flavanoids	Sterols and terpenes	Quinons	Saponins	Terpenoids	Coumarins	Anthra-quinones	Reducing sugars	Alkaloids		Tannins
										mayer	wagner	
<b>Aerial part</b>	AQ	-	++	+	-	+	+	-	+++	-	-	+++
	CL	++	+++	+	-	+	++	+	+++	+++	-	+
	AQ-ME	+++	+++	+	-	+++	+++	-	+++	-	-	+++
	AQ-AC	-	+	+	-	+	+++	-	+	+	+	+++
<b>Root</b>	AQ	-	-	-	-	+	++	-	+	-	-	-
	CL	++	+++	+	-	++	++	-	+++	+++	++	+
	AQ-ME	+	++	+	-	+	+++	-	+++	+	-	++
	AQ-AC	+	++	+	-	+	+++	-	+	-	-	++

AQ: Aqueous extract, CL: Chloroform extract, AQ- ME: Aqueous- Methanol extract, AC: Aqueous -Acetone extract. - = Absent, + = Present, ++ = Moderately present, +++ = Extremely present

**Table 2:** Antibacterial activity of aerial part extracts of *P. scoparius*.

Organisms	Diameter of zone of inhibition (mm)					
	Aerial part extracts (200 mg/ml)				Antibiotics	
	AQ	CL	AQ-ME	AQ-AC	GENT	CEF
<i>Staphylococcus aureus</i> ATCC 25923	NA	NA	12±1.73	7±0	22.66±1.15	27.5±0.5
methicillin-resistant <i>Staphylococcus aureus</i> MRSA ATCC 43300	NA	8.66±0.57	7.66±0.57	7±0	17.66±1.15	24.33±0.57
<i>Bacillus cereus</i> ATCC11778	NA	10±1	<b>11.83±0.28</b>	<b>14.66±0.57</b>	12.66±0.57	9.83±0.28
<i>Bacillus subtilis</i> ATCC 6633	NA	9.33±0.57	<b>15±1</b>	<b>12.66±0.57</b>	13.66±0.57	11.16±0.28
<i>Enterococcus faecalis</i> ATCC 25212	NA	NA	9.33±0.57	6.66±0.57	16.33±0.57	8±0
<i>Listeria monocytogenes</i> ATCC 19115	NA	NA	<b>13.66±0.57</b>	7.25±0.35	11±1	8.33±0.57
<i>Enterobacter cloacae</i> ATCC 13047	NA	6.66±0.57	<b>11.5±0.5</b>	8.83±0	16.83±1.04	7.33±0.57
<i>Acinetobacter baumannii</i> ATCC 19606	NA	NA	NA	8.66±0.57	13.33±0.57	15.66±0.57
<i>Klebsiella pneumoniae</i> ATCC 70603	NA	NA	<b>11.5±0.86</b>	6.33±0.57	14.33±1	17.16±0.28
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.33±0.57	8.66±0.57	<b>10.33±1.15</b>	<b>17±0</b>	20.66±1.15	19.33±0.57
<i>Proteus mirabilis</i> ATCC 35659	6.66±0.57	NA	NA	<b>22.33±0.57</b>	24.33±0.57	25.83±1.04
<i>Salmonella</i> Typhimurium ATCC 13311	6.33±0.57	NA	<b>11±0.86</b>	NA	25.5±0.70	19.16±0.76
<i>Escherichia coli</i> ATCC 25933	NA	NA	6.33±0.57	6.33±0.57	13.66±1.15	15.66±0.57
<i>Citrobacter freundii</i> ATCC 8090	NA	6.66±0.57	<b>10±0</b>	6.33±0.57	28.66±1.15	14.33±0.57

AQ: Aqueous extract, CL: Chloroform extract, AQ- ME: Aqueous- Methanol extract, AC: Aqueous -Acetone extract, NA: no activity, GET: Gentamicin, CEF: Cephotaxime.

**Table 3:** Antibacterial activity of root part extracts of *P. scoparius*.

Organisms	Diameter of zone of inhibition (mm)					
	Root extracts (200 mg/ml)				Antibiotics	
	AQ	CL	AQ-ME	AQ-AC	GENT	CEF
<i>Staphylococcus aureus</i> ATCC 25923	NA	NA	8.33±0.57	NA	22.66±1.15	27.5±0.5
methicillin-resistant <i>Staphylococcus aureus</i> MRSA ATCC 43300	NA	<b>10.33±1.52</b>	<b>10±1</b>	NA	17.66±1.15	24.33±0.57
<i>Bacillus cereus</i> ATCC11778	NA	NA	9.33±0.57	9.33±0.57	12.66±0.57	9.83±0.28
<i>Bacillus subtilis</i> ATCC 6633	NA	NA	6.66±0.57	<b>11±1</b>	13.66±0.57	11.16±0.28
<i>Enterococcus faecalis</i> ATCC 25212	NA	NA	<b>10.33±0.57</b>	NA	16.33±0.57	8±0
<i>Listeria monocytogenes</i> ATCC 19115	NA	7.66±0.57	NA	NA	11±1	8.33±0.57
<i>Enterobacter cloacae</i> ATCC 13047	NA	NA	NA	NA	16.83±1.04	7.33±0.57
<i>Acinetobacter baumannii</i> ATCC 19606	NA	NA	NA	NA	13.33±0.57	15.66±0.57
<i>Klebsiella pneumoniae</i> ATCC 70603	NA	NA	NA	NA	14.33±1	17.16±0.28
<i>Pseudomonas aeruginosa</i> ATCC 27853	NA	<b>12.5±0.86</b>	NA	NA	20.66±1.15	19.33±0.57
<i>Proteus mirabilis</i> ATCC 35659	NA	NA	NA	NA	24.33±0.57	25.83±1.04
<i>Salmonella</i> Typhimurium ATCC 13311	NA	NA	NA	NA	25.5±0.70	19.16±0.76
<i>Escherichia coli</i> ATCC 25933	NA	NA	NA	NA	13.66±1.15	15.66±0.57
<i>Citrobacter freundii</i> ATCC 8090	NA	9.33±0.57	NA	NA	28.66±1.15	14.33±0.57

AQ: Aqueous extract, CL: Chloroform extract, AQ- ME: Aqueous- Methanol extract, AC: Aqueous -Acetone extract, NA: no activity, GET: Gentamicin, CEF: Cephotaxime.

**Table 4:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of the aerial part of *P. scoparius* using different solvents (mg/ml).

Organisms	Aerial part extracts					
	MIC			MBC		
	CL	AQ-ME	AQ-AC	CL	AQ-ME	AQ-AC
<i>Staphylococcus aureus</i> ATCC 25923	3.12±2.70	6.25 ±3.60	2.6±0.90	3.12±2.70	12.5±0	16.66±7.21
methicillin-resistant <i>Staphylococcus aureus</i> MRSA ATCC 43300	12.5±0	4.16±1.80	12.5±0	25±0	8.33±3.6	25±0
<i>Bacillus cereus</i> ATCC11778	6.25±0	12.5±0	4.16±1.80	6.25±0	50±0	8.33±3.6
<i>Bacillus subtilis</i> ATCC 6633	6.25±0	6.25±0	3.12±0	6.25±0	16.66±7.21	5.20±1.8
<i>Enterococcus faecalis</i> ATCC 25212	6.25±0	1.56± 1.35	8.33±3.60	6.25±0	3.12±2.70	12.5±10.82
<i>Listeria monocytogenes</i> ATCC 19115	12.5±0	25±0	12.5±0	50±0	50±0	25±0
<i>Enterobacter cloacae</i> ATCC 13047	50±0	12.5±0	4.68±2.70	50±0	16.66±7.21	12.5±0
<i>Acinetobacter baumannii</i> ATCC 19606	6.25±0	3.12±0	9.37±5.41	8.33±3.6	3.12±0	17.70±12.62
<i>Klebsiella pneumoniae</i> ATCC 70603	25±0	12.5±0	6.25±0	50±0	12.5±0	6.25±0
<i>Pseudomonas aeruginosa</i> ATCC 27853	25±0	12.5±0	3.12±0	50±0	25±0	12.5±0
<i>Proteus mirabilis</i> ATCC 35659	12.5±10.82	6.25±0	4.16±1.80	25±0	12.5±10.82	50±0
<i>Salmonella</i> Typhimurium ATCC 13311	50±0	8.33±3.6	1.04±0.45	50±0	8.85±6.31	1.56±1.35
<i>Escherichia coli</i> ATCC 25933	12.5±0	4.16±1.80	>50	50±0	12.5±0	>50
<i>Citrobacter freundii</i> ATCC 8090	16.66±7.21	16.66±7.21	12.5±0	25±0	20.83±7.21	12.5±0

AQ: Aqueous extract, CL: Chloroform extract, AQ- ME: Aqueous- Methanol extract, AC: Aqueous -Acetone extract.

**Table 5:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts of the root part of *P. scoparius* using different solvents(mg/ml).

Organisms	Root extracts					
	MIC			MBC		
	CL	AQ-ME	AQ-AC	CL	AQ-ME	AQ-AC
<i>Staphylococcus aureus</i> ATCC 25923	12.5±0	16.66±7.21	25±0	16.66±7.21	33.33±14.43	41.66±14.43
methicillin-resistant <i>Staphylococcus aureus</i> MRSA ATCC 43300	20.83±7.21	8.33±3.60	50±0	25±0	25±0	50±0
<i>Bacillus cereus</i> ATCC11778	25±0	33.33±14.43	25±0	25±0	50±0	25±0
<i>Bacillus subtilis</i> ATCC 6633	20.83±7.21	50±0	50±0	41.66±14.43	50±0	50±0
<i>Enterococcus faecalis</i> ATCC 25212	20.83±7.21	25±0	41.66±14.43	41.66±14.43	33.33±14.43	41.66±14.43
<i>Listeria monocytogenes</i> ATCC 19115	20.83±7.21	25±0	25±0	50±0	25±0	25±0
<i>Enterobacter cloacae</i> ATCC 13047	20.83±7.21	8.33±3.60	33.33±14.43	25±0	25±0	50±0
<i>Acinetobacter baumannii</i> ATCC 19606	16.66±7.21	6.25±0	25±0	25±0	6.25±0	33.33±14.43
<i>Klebsiella pneumoniae</i> ATCC 70603	20.83±7.21	50±0	41.66±14.43	25±0	50±0	41.66±14.43
<i>Pseudomonas aeruginosa</i> ATCC 27853	20.83±7.21	33.33±14.43	50±0	25±0	33.33±14.43	50±0
<i>Proteus mirabilis</i> ATCC 35659	25±0	50±0	50±0	25±0	50±0	50±0
<i>Salmonella</i> Typhimurium ATCC 13311	20.83±7.21	6.25±0	33.33±14.43	20.83±7.21	25±0	50±0
<i>Escherichia coli</i> ATCC 25933	>50	25±0	25±0	>50	25±0	25±0
<i>Citrobacter freundii</i> ATCC 8090	16.66±7.21	25±0	25±0	50±0	25±0	50±0

AQ: Aqueous extract, CL: Chloroform extract, AQ- ME: Aqueous- Methanol extract, AC: Aqueous -Acetone extract.

**Table 6:** Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of antibiotics reference (mg/ml).

Organisms	Antibiotics			
	MIC		MBC	
	GNT	CEF	GNT	CEF
<i>Staphylococcus aureus</i> ATCC 25923	0.19±0	25±0	0.19±0	25±0
methicillin-resistant <i>Staphylococcus aureus</i> MRSA ATCC 43300	0.39±0	12.5±0	1.56±0	25±0
<i>Bacillus cereus</i> ATCC11778	0.19±0	50±0	0.19±0	50±0
<i>Bacillus subtilis</i> ATCC 6633	5.20±1.80	>50	6.25±0	>50
<i>Enterococcus faecalis</i> ATCC 25212	0.78±0	>50	0.78±0	>50
<i>Listeria monocytogenes</i> ATCC 19115	2.21±1.57	6.25±0	2.21±1.57	12.5±0
<i>Enterobacter cloacae</i> ATCC 13047	2.60±0.90	>50	5.20±1.8	>50
<i>Acinetobacter baumannii</i> ATCC 19606	0.65±0.22	6.25±0	0.78±0	6.25±0
<i>Klebsiella pneumoniae</i> ATCC 70603	4.16±1.80	>50	8.33±3.6	>50
<i>Pseudomonas aeruginosa</i> ATCC 27853	0.78±0	25±0	1.56±0	50±0
<i>Proteus mirabilis</i> ATCC 35659	0.19±0	8.33±3.60	0.19±0	12.5±10.82
<i>Salmonella</i> Typhimurium ATCC 13311	0.65±0.22	10.41±3.60	0.65±0.22	18.75±10.82
<i>Escherichia coli</i> ATCC 25933	0.32±0.11	12.5±0	0.32±0.11	25±0
<i>Citrobacter freundii</i> ATCC 8090	0.19±0	6.25±0	0.19±0	12.5±0

GET: Gentamicin, CEF: Cephotaxime

## DISCUSSION

Over the past decade, there has been an explosion of interest in the antimicrobial, particularly antibacterial and antifungal, activities of natural products (Melissa and Larry, 2005). This was motivated by a number of factors, including the increasing antibiotic resistance and fear of development of even more infectious “superbugs”, the impact of infectious diseases on mortality and morbidity, the growing interest in “natural” therapies and a move to more self-care. Plants are good sources of novel antimicrobial chemotherapeutic agents (Shahbudin et al., 2011). Undoubtedly, the plant kingdom still hold many species that contain substances of medicinal value, which are yet to be discovered, though a lot of plants are constantly being screened for their antimicrobial properties (Jahir and Saurabh, 2011). Furthermore, plant extracts and products are used in the treatment of infectious diseases (Qaralleh et al., 2009; Lee and Lee, 2010). Plants may prove to be a rich source of a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids and flavonoids, with possible antimicrobial properties (Burt and Reinders, 2003).

A number of review and research articles provide interesting information of the biological activities of plants of the Apiaceae family. They possess a wide range of compounds with many biological activities. Some of their main properties are the ability to induce apoptotic, antibacterial, antimicrobial, antifungal, anti-inflammatory, antioxidant, hepatoprotective activities and antitumor actions (Oroojalian et al., 2010; Asili et al., 2009).

In the present study, an attempt was made to screen various solvent extracts for their antibacterial activities against several bacteria. Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against predation by many

microorganisms, insects and herbivores (Lutterodt et al., 1999; Marjorie, 1999). This may therefore explain the presentation of antimicrobial activity by the *Pituranthos scoparius*. The demonstration of antibacterial activity against both Gram positive and Gram negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds (Srinivasan et al., 2001). This will be of immense interest in fighting the menace of antibiotic refractive pathogens that have been so prevalent in recent times.

Different solvents were reported to have the capacity to extract numerous phytoconstituents, depending on their solubility or the polarity of the solvent (Marjorie, 1999). Acetone is routinely selected as a solvent to prepare extracts for the initial screening process. Just like several other studies solvents, acetone was found to yield the best results with reference to quantity and diversity of compounds extracted, number of inhibitors extracted, toxicity in a bioassay, and ease of removal of solvent, among other factors (Eloff, 1998).

The extracts from aerial parts showed greater antibacterial activities than those from roots. It is likely that interactions between various compounds of secondary metabolites, present in the extracts, result in synergistic effects which lead to heightened activity (Williamson, 2001). There is a distinct possibility that active principles, with differing mechanisms of action, may be present in a crude extract, thus slowing the onset of antibiotic resistance.

Boutaghane et al. (2004) reported a comparative study on the antibacterial activities of essential oils from stems and seeds of species collected from the region of Ghardaia (Algeria). These two kinds of essential oils inhibited the growth of the tested strains, but the seed essential oil showed lower MIC values with *Proteus mirabilis*, *Pseudomonas aeruginosa* ATCC27853, and *Staphylococcus aureus* ATCC25923 at 0.156 mg/ml; 1.25 mg/ml, and 20 mg/ml respectively.

Benmekhbi et al. (2008), noted in their study that the butanolic extract from *Pituranthos scoparius*, using the disk diffusion method, showed good antibacterial activity against microorganisms like *Escherichia coli* ATCC 25922 (30 mm), *Pseudomonas aeruginosa* ATCC 27853 (30 mm), and *Staphylococcus aureus* (30 mm), with the respective minimal inhibition concentration levels 0.03 µg/ml; 0.125 µg/ml, and 8 µg/ml.

The antibacterial activity was more pronounced on the Gram positive bacteria than the Gram negative bacteria species. The reason for the difference in sensitivity between Gram positive and Gram negative bacteria might be ascribed to the differences in the morphological constitutions of these microorganisms; Gram negative bacteria have an outer phospholipidic membrane possessing structural lipopolysaccharide components (Pitchamuthu, 2012). This makes the cell wall impermeable to antimicrobial chemical substances. On the other hand, Gram positive bacteria are more susceptible, having only a peptidoglycan layer which is not an effective permeability barrier (Nostro et al., 2000; Sharma et al., 2010).

### Conclusion

*Pituranthos scoparius* exhibits broad spectrum of antibacterial activities and this may help discover new chemical classes of antibiotic substances that could serve as selective antibiotic agents against many infectious diseases. The effect of *P. scoparius* on more pathogenic organisms needs to be investigated; additional toxicological studies and purification of its active components require more attention.

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