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Effectiveness of *Passiflora foetida* (Baby Semitoo) and *Ocimum campechianum* extracts (Married Man Pork) against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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Abstract. *Scott S, Daniel R, Kalicharran L. 2023. Effectiveness of* Passiflora foetida *(Baby Semitoo) and* Ocimum campechianum *extracts (Married Man Pork) against* Pseudomonas aeruginosa *and* Klebsiella pneumoniae*. Asian J Nat Prod Biochem 21: 79-87.* Medicinal plants have long been used to treat illnesses. They contain many secondary metabolites, which have antibiotic properties, and can treat antimicrobial resistance in bacteria. The aim of this study was to investigate the phytochemicals in *Passiflora foetida* L. (Baby Semitoo) and *Ocimum campechianum* Mill. (Married Man Pork) and to analyze the antimicrobial potential against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Plant extracts were prepared using hexane, methanol, and water, with a rotary evaporator. The extracts were tested using zone of inhibition, Minimum Inhibitory Concentration (MIC), and Minimum Bacterial Concentration (MBC) methods. The extract of *O. campechianum* produced the highest percent yield for each solvent, while methanol produced the highest yield of all solvents. The results of the phytochemical test showed that, *O. campechianum* contained flavonoids, tannins, saponins, glycosides, and phenols; while flavonoids, tannins, glycosides, and saponins were found in *P. foetida* extract. Similarly, *O. campechianum* also showed higher antimicrobial potential than *P. foetida*. The *P. aeruginosa* proved more susceptible than *K. pneumoniae* to both plants.

Keywords: Antibiotic resistance, *Klebsiella pneumonia, Ocimum campechianum, Passiflora foetida*, *Pseudomonas aeruginosa*

Abbreviations: MBC: Minimum Bacterial Concentration, MDR: Multidrug-Resistant, MIC: Minimum Inhibitory Concentration

INTRODUCTION

The ability of bacteria to withstand the antagonizing effect of an antibacterial agent upon reproduction prevention or bactericidal is known as resistance. Antibiotic resistance develops because of inappropriate and unregulated usage of antibiotics (Cesur and Demiröz 2013). The intense use of antibiotics has resulted in the evolution of resistant microorganisms over the years. When antibiotics fail to eliminate all pathogens, those surviving become stronger and more resistant. The resultant resistance is then spread rapidly between the organisms either by horizontal transfer or cell division (Rossiter et al. 2017).

Before the advent of antibiotics, illnesses were treated with natural, home remedies. Treatment spanned from the mending of minor scrapes and bruises to serious ailments like pneumonia and asthma. The effectiveness of home treatments is evident because humans have continued to survive plagues, and all other ailments that occurred in the past (Ionescu 2018).

The two plants used in this study were *Passiflora foetida* L. (Baby Semitoo) and *Ocimum campechianum* Mill. (Married Man Pork). The *P. foetida* commonly called Baby Semitoo, belongs to the family Passifloraceae (DeFilipps et al. 2004). *Passiflora*, also known as passion flower or passion vine, consists of an average 500 species that can be found in temperate and tropical regions. It includes dicotyledonous shrubs, climbers and herbs. Species of Passiflora may contain indole alkaloids such as harmane, harmaline, and harmol; flavonoids such as apigenin, luteolin, and scopoletin; or benzoflavone. The flower and fruit have only traces of these chemicals, however, the leaves and the roots are potent (Tiwari et al. 2015). The *P. foetida* contains cyanogenic glycosides, gynocardin, and flavonoids.

The *O. campechianum* commonly known as Married Man Pork, belongs to the family Lamiaceae (DeFilipps et al. 2004). Lamiaceae (mint), includes 236 genera and over 7,000 species. Species of mint may contain flavonoids and sterols. They can also be classified into several biological categories such as antioxidant, cytotoxic, antibacterial, antifungal and antiviral (Nousiba et al. 2020). The *O. campechianum* leaves can soothe colic, reduce tumors, remedy swollen groin, and treat red sediments in urine, while the seeds may be used to treat the irritated eyes of children. The plant contains three chemical compounds: eugenol, methyl eugenol, and sesquiterpenes (DeFilipps et al. 2004).

The aforementioned plants were tested against two known resistant microbes, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae,* which can cause hospital-acquired and respiratory infections respectively. The *P. aeruginosa* is a gram-negative bacillus that can be found in freshwater and community reservoirs. Infections include folliculitis,

pneumonia, and otitis externa (Wilson and Pandey 2023). The *P. aeruginosa* is opportunistic and causes infections, such as ventilator-associated pneumonia and catheter-associated urinary tract infections. Infections are usually severe, and treatment can be difficult because the bacterium has a limited susceptibility to antimicrobials (Reynolds and Kollef 2021). Its antibiotic resistance is expressed by restricting membrane permeability, efflux systems, and antibiotic-inactivating enzymes (Wilson and Pandey 2023).

In contrast, *K. pneumoniae* contributes to 11.8% of all hospital-acquired pneumonia worldwide. It is highly resistant and poses a significant threat to immunocompromised patients. The *K. pneumoniae* is a gram-negative bacterium of the Enterobacteriaceae family. The pathogen has a high infection and low recovery rate. Its antibiotic resistance is expressed by mechanisms of evasion, such as usage of porins and efflux pumps (Paczosa and Mecsas 2016; Ashurst and Dawson 2023).

The study investigates possible native plants that can be used to treat two pathogens that cause great harm to human health. Respiratory infections are infectious diseases of the upper and lower respiratory tract, and are transmitted by bacteria, viruses, and fungi (Saleri and Ryan 2019). These pathogens have a great impact on human health because they can cause illnesses and also increase the effects of chronic lung diseases. In 2015, respiratory infections were the cause of 3.2 million deaths, and in low-income countries they are one of the leading causes of deaths (Jose 2018). In contrast, hospital-acquired infections are nosocomial infections that occur while receiving health care. Worldwide about 1.7 million persons acquire these infections and over 98,000 persons die yearly. Generally, immunocompromised patients are most susceptible to infections (Haque et al. 2018).

This study investigated the effectiveness of phytochemicals extracts in *P. foetida* and *O. campechianum* and analyzed their antimicrobial potential against *K. pneumoniae* and *P. aeruginosa.*

MATERIALS AND METHODS

Experimental design

The experiment was based on the randomized block design, whereby the two medicinal plants *P. foetida* and *O. campechianum* were tested against two pathogens *P. aeruginosa* and *K. pneumoniae* at concentrations of 0.05g/mL, 0.1g/mL, and 0.15g/mL.

Sample collection

The two medicinal plants, namely *P. foetida* and *O. campechianum* were bought from the local market and microbes *P. aeruginosa* and *K. pneumoniae* were obtained from the George Town Public Hospital Corporation (GPHC) laboratory and sub-cultured weekly to maintain the colonies. The experiments were conducted in the Microbiology and Quality Control Laboratories of the New Guyana Pharmaceutical Company, and the Plant Pathology Laboratory at the National Agricultural Research and Extension Institute (NAREI). Experimentation began in May and was concluded in June 2021.

Preparation of plant extract

The leaves of each plant were rinsed carefully to remove dust and soil particles. Leaves were cut into small pieces using a sterile pair of scissors and 50 g of each plant sample was soaked in 500 mL of each solvent (distilled water, methanol, and hexane). This mixture was left at room temperature for eleven days, during which occasional shaking was done to increase extraction. The mixtures were filtered using Whatman No.1 filter paper and methanol and hexane extracts were dried using a rotary evaporator. The aqueous *P. foetida* and *O. campechianum* extracts were dried using a hot plate until a small quantity of liquid remained, then further drying was done in a hot air oven. After drying all extracts were carefully labeled and stored in a dry place until further investigation (Shahid et al. 2013). The percentage yield of each extract was calculated using the formula:

Yield (%) = $(X_1*100)/X_0$ where X_1 refers to the weight of extract after evaporation of the solvent and X_0 refers to the dry weight of plant before extraction (Gonelimali et al. 2018).

Phytochemical analysis

Phytochemical analysis, included terpenoids, flavonoids, glycosides, tannins, saponins, phlobatannins, coumarins, phenols, quinones, triteroids, and steroids (Table 1).

Microbial analysis

The zone of inhibition test was done in triplicates, while the other two tests were performed in duplicates. In these three tests, methanol and distilled water were used as the negative controls for methanolic and aqueous extracts, respectively. For the positive control, the antibiotic ciprofloxacin was used.

Sterilization

All equipment was sterilized in an autoclave before use, such as the test tubes, borer, and tweezers. The agar plates were sterilized before use for two and a half hours in a hot air oven.

0.5 McFarland standard

The solution was prepared by mixing 9.95 ml of 1% H_2SO_4 with 0.05 ml of 1% $BaCl_2$. The solution was stored in an Erlenmeyer flask with a glass stopper and used to standardize the bacterial suspensions (De Zoysa 2019).

Broth preparation

Tryptic soy broth was prepared according to the instructions on the container. Then after autoclaving, broth was pipetted into the required test tubes for usage.

Agar preparation

Mueller Hinton agar was prepared according to the instructions on the container. Then after autoclaving, agar was poured into sterilized agar plates. Plates were stored in the refrigerator until ready to use.

Table 1. Procedures of testing phytochemical compounds present in the plants (Thilagavathi et al. 2015; Roghini and Vijayalakshmi 2018)

Phytochemical	Methodology	Indicator
Terpenoids	0.8 g of each plant extract was transferred to a test tube and 10 mL of methanol was added to the tube. The mixture was filtered, and 5 mL of the filtrate was removed. 5 mL of the solution was transferred to another test tube and 2 mL of chloroform and 3 mL of sulfuric acid were added.	Reddish-brown color
Flavonoids	0.5 g of each plant extract was added to a test tube with 10 mL of distilled water. After mixing, 5 mL of dilute ammonia solution, then 1 mL of concentrated H_2S0_4 were added.	Yellow color
Glycosides	A few drops of glacial acetic acid and ferric chloride were added to 1 mL of each extract. Then 3-4 drops of concentrated sulfuric acid were also added to each extract.	Blue-green color
Tannins	A few drops of 10% lead acetate were added to 2 mL of each extract.	White precipitate
Saponins	9 mL of distilled water was added to 1 mL of each extract and shaken vigorously for 15 seconds. The extract was then allowed to stand for 10 minutes.	Stable foam
Triteroids	To 2 mL of each extract was added 10 mL of chloroform. After which 1 mL of acetic anhydride and 2 mL of concentrated sulfuric acid were also added.	Red, pink, or violet color
Phlobatannins	A few drops of 2% hydrochloric acid were added to 1 mL of each extract.	Red precipitate
Coumarins	To 1 mL of each extract was added 1 mL of 10% sodium hydroxide.	Yellow color
Phenols	To 1 mL of each extract, 2 mL of distilled water and a few drops of 10% ferric chloride were added.	Blue or green color
Quinones	1 mL of concentrated sulphuric acid was added to 1 mL of each extract.	Red color
Steroids	To 2 mL of each extract was added 10 mL of chloroform. After which 1 mL of acetic anhydride and 2 mL of concentrated sulfuric acid were also added.	Blue-green color

Inoculum preparation

The inoculum was prepared using the direct colony suspension method. 5 mL of saline was poured into a test tube and sterilized for 15 minutes in an autoclave. After cooling, bacterial suspension was prepared by transferring 2-3 small colonies from the 24-hour culture to the test tubes. The inoculum was standardized using the 0.5 McFarland standard prepared previously.

Agar well-diffusion test

Extracts were made using 0.15 g/mL, 0.1 g/mL and 0.05 g/mL crude extracts in the respective solvents. The Mueller Hinton agar plates were inoculated by swabbing the organism on the surface of agar plate using sterile cotton swabs (six plates for each organism). After which 6 mm wells (three wells per plate) were made using a sterile laboratory cork borer and tweezers. The wells were then filled with 30-40 µL of each extract using 1 mm syringes (one per well). The agar plates were incubated for 18-24 hours, and the zone of inhibition for each well was measured (Balouiri et al. 2016).

Minimum Inhibitory Concentration (MIC) using agar dilution

Plant extracts were tested for MIC using the highest concentration of extracts (0.15 g/mL). For this 1:9 dilution was made for each of the four extracts. 2 mL of the extract was added to test tube 1 and 1 mL of sterile Tryptic Soy Broth (TSB) was added to the other nine tubes. Using a 1 mL glass pipette and pipette filler, 1 mL of the extract from test tube 1 was transferred to test tube 2. Then after mixing, 1 mL of test tube 2 was added to test tube 3. The dilutions were continued until test tube 9 where 1 mL was discarded. From a 0.5 McFarland standard TSB culture suspension of the organism, 1 mL of the culture suspension was added to each test tube. This test was then repeated for the other organism. After which the tubes were incubated for 18-24 hrs and the turbidity of each test tube was observed. Any turbidity in the tubes suggested growth. The smallest dilution with no growth was the MIC (Rollins 2000, unpublished data).

Minimum Bacterial Concentration (MBC)

The MBC was determined by inoculating the dilutions used for MIC, showing no growth on the surface of agar plates. This was done using Mueller Hinton agar plates and the plates were inoculated for 18-24hrs at 37°C. The plates were divided into quarters and for each test tube that didn't display visible growth, streaking was done using an inoculating loop. Any growth of colonies indicated that plant extract was bacteriostatic but not bactericidal, while the absence of growth indicated it was bactericidal. The test was performed for both pathogens using both plant extracts (Rollins 2000).

Data analysis

The results obtained consisted of both quantitative and qualitative variables. Descriptive and inferential statistics were conducted to analyze the results. Using descriptive statistics, histograms and bar charts were generated, along with the means and standard deviations. While using inferential statistics, one way ANOVA and the Tukey test were conducted on the raw data. Analysis was done using SPSS and Excel software.

RESULTS AND DISCUSSION

Yield preparation

The amount and percentage yield from each plant using each solvent is shown in Table 2. For *P. foetida*, water extract produced the highest yield while for *O. campechianum*, methanol produced the highest yield. Hexane produced no yield for both plant samples.

Table 2. Calculation of the % yield of the extracts of *P. foetida and O. campechianum*

Plants	Solvents	Weight before extraction (g)	Weight after drying (g)	Yield (%)
P. foetida	Methanol	50	1.411	2.82
	Water	50	1.97	3.94
	Hexane	50	0	0
O. campechianum	Methanol	50	2.606	5.21
	Water	50	1.225	2.45
	Hexane	50	0	0

Phytochemical analysis

The phytochemicals present in methanolic, and aqueous extracts of each plant are shown in Table 3. Glycosides were present in aqueous and methanolic extracts of both plants. Flavonoids, tannins, glycosides, and phenols were present in the methanolic extract of *O. campechianum*, while flavonoids, tannins, and glycosides were present in methanolic extract of *P. foetida*. The aqueous extract of *O. campechianum* was found to contain tannins, glycosides, and saponins, while aqueous extract of *P. foetida* only contained glycosides and saponins.

Table 3. Plant compounds present in *P. foetida* and *O. campechianum* extracts

Plant compounds	*O. campechianum*		*P. foetida*	
	Methanolic	Aqueous	Methanolic	Aqueous
Terpenoids	-	-	-	-
Flavonoids	+	-	+	-
Glycosides	+	+	+	+
Tannins	+	+	+	-
Saponins	-	+	-	+
Phlobatannins	-	-	-	-
Coumarins	-	-	-	-
Phenols	+	-	-	-
Quinones	-	-	-	-
Steroids	-	-	-	-
Triteroids	-	-	-	-

Note: (-) Absence; (+) Presence

Microbial analysis

Agar well diffusion for P. aeruginosa

The zone of inhibition recorded for *P. foetida* was shown at three concentrations (0.15 g/mL, 0.05 g/mL, and 0.1 g/mL) against *P. aeruginosa*. Zones of inhibition were observed for two concentrations (0.1 g/mL and 0.15 g/mL) of methanolic extract indicating only methanolic *P. foetida* extract can inhibit growth of *P. aeruginosa*. The largest zone of inhibition occurred at 0.15 g/mL for methanolic *P. foetida* extract (Table 4).

The zone of inhibition recorded for *O. campechianum* was shown at three concentrations (0.15 g/mL, 0.05 g/mL, and 0.1 g/mL) against *P. aeruginosa*. Zones of inhibition were observed for both extracts at all tested concentrations indicating *P. aeruginosa* is susceptible to *O. campechianum*. The largest zone of inhibition occurred at 0.15 g/mL for aqueous extract and 0.1 g/mL for methanolic extract (Table 4).

The mean zone of inhibition for each extract at the three concentrations is shown in Figure 1. The results show that aqueous *O. campechianum* extract produced the largest zone of inhibition for all extracts against *P. aeruginosa*. The results of aqueous *P. foetida* can be seen at the blue bars (0 mm) indicating no zone of inhibition was observed.

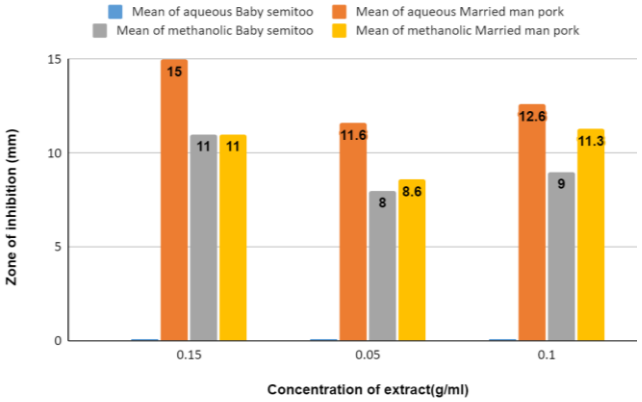

Figure 1. Mean zone of inhibition for plant extracts against *P. aeruginosa*

Table 4. Zone of inhibition in *P. foetida* and *O. campechianum* extracts

Concentrations of extract (g/mL)	Methanol				Aqueous			
	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean±SD	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean±SD
P. foetida								
0.05	8	8	-	8±5.6	-	-	-	-
0.1	9	10	8	9±0.7	-	-	-	-
0.15	11	10	12	11±0.7	-	-	-	-
O. campechianum								
0.15	17	15	13	15±2.8	12	11	10	11±1.4
0.05	13	10	12	11.6±0.7	10	8	8	8.6±1.4
0.1	13	11	14	12.6±0.7	11	11	12	11.3±1.4

Note: (-) no zone of inhibition

Agar well diffusion for K. pneumoniae

The zone of inhibition for *P. foetida* was recorded at three concentrations (0.15 g/mL, 0.05 g/mL, and 0.1 g/mL) against *K. pneumoniae*. Zones of inhibition were not observed for neither methanolic nor aqueous extracts indicating *K. pneumoniae* was resistant to *P. foetida* (Table 5).

The zone of inhibition for *O. campechianum* was recorded at three concentrations (0.15 g/mL, 0.05 g/mL, and 0.1 g/mL) against *K. pneumoniae*. Zones of inhibition were observed for two concentrations (0.1 g/mL and 0.15 g/mL) of aqueous extract indicating aqueous *O. campechianum* extract can inhibit the growth of *K.pneumoniae*. The same zone of inhibition was observed at both concentrations (Table 5).

The mean zone of inhibition for each extract at the tested concentrations is shown in Figure 2. Results indicate that only aqueous *O. campechianum* extract can inhibit *K. pneumoniae*.

The zone of inhibition for the antibiotic ciproflaxacin at 0.1 g/mL against *P. aeruginosa* and *K. pneumoniae* is shown in Table 6.

Minimum Inhibitory Concentration (MIC)

The results of minimum inhibitory concentration for the extracts of *O. campechianum* against *K. pneumoniae* and *P. aeruginosa* are presented in Table 7. The results below indicate that methanolic extract cannot inhibit growth of *K. pneumoniae* and the pathogen is resistant to the extract. The MIC of methanolic extract against *P. aeruginosa* was 38 mg/mL (10^{-3}). The MIC of aqueous extract against *P. aeruginosa* was19 mg/mL (10^{-4}), while MIC against *K. pneumoniae* was 150 mg/mL (10^{-1}).

The Minimum Inhibitory Concentration (MIC) of methanolic and aqueous extracts of *P. foetida* against *P. aeruginosa* and *K. pneumoniae* is presented in Table 7. The results indicate that neither methanolic nor aqueous extract can inhibit growth of *K. pneumoniae* and the pathogen was resistant to *P. foetida*. The MIC of methanolic *P. aeruginosa* was 38 mg/mL (10^{-3}), while no inhibition was observed for aqueous *P. foetida* extract.

Minimum Bacterial Concentration (MBC)

The results of Minimum Bacterial Concentration (MBC) of methanolic and aqueous extract of *P. foetida* are shown in Table 8. It was observed that methanolic extract can kill *P. aeruginosa*, while aqueous extract cannot. The MBC of methanolic extract was 75mg/mL(10^{-2}). It was observed that neither methanolic nor aqueous extract can kill *K. pneumoniae.*

The Minimum Bacterial Concentration (MBC) of methanolic and aqueous extracts of *O. campechianum* are shown in Table 8. The results indicate that *O. campechianum* cannot kill either *P. aeruginosa* or *K. pneumoniae*. Neither methanolic nor aqueous extracts can kill both pathogens.

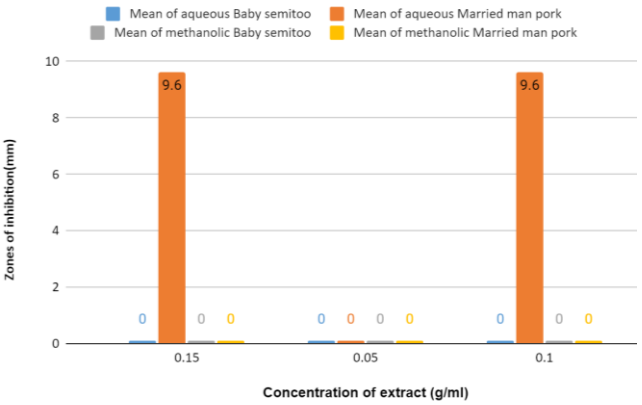

Figure 2. Mean zone of inhibition in plant extracts against *K. Pneumoniae*

Table 6. Zone of inhibition obtained from antibiotic ciprofloxacin

Microbes	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean±SD
K. pneumoniae	41	38	42	40.3±2.8
P. aeruginosa	52	50	48	50 ±2.8

Table 5. Zone of inhibition for *P. foetida* and *O. campechianum* extracts

Concentrations of Extract (g/mL)	Aqueous				Methanol			
	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean±SD	Trial 1 (mm)	Trial 2 (mm)	Trial 3 (mm)	Mean
P. foetida								
0.05	-	-	-	-	-	-	-	-
0.1	-	-	-	-	-	-	-	-
0.15	-	-	-	-	-	-	-	-
O. campechianum								
0.05	-	-	-	-	-	-	-	-
0.1	11	10	8	9.6±2.1	-	-	-	-
0.15	10	10	9	9.6±2.1	-	-	-	-

Note: (-) no zone of inhibition.

Table 7. MIC of methanolic and aqueous extracts of *P. foetida* and *O. campechianum*

Dilutions	Concentrations (mg/mL)	*P. aeruginosa*		*K. pneumoniae*	
		Methanolic Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract
P. foetida					
10^{-1}	150	-	+	+	+
10^{-2}	75	-	+	+	+
10^{-3}	38	-	+	+	+
10^{-4}	19	+	+	+	+
10^{-5}	9	+	+	+	+
10^{-6}	5	+	+	+	+
10^{-7}	2	+	+	+	+
10^{-8}	1	+	+	+	+
10^{-9}	0.5	+	+	+	+
O. campechianum					
10^{-1}	150	-	-	-	+
10^{-2}	75	-	-	+	+
10^{-3}	38	-	-	+	+
10^{-4}	19	+	-	+	+
10^{-5}	9	+	+	+	+
10^{-6}	5	+	+	+	+
10^{-7}	2	+	+	+	+
10^{-8}	1	+	+	+	+
10^{-9}	0.5	+	+	+	+

Note: (-) No growth; (+) Growth

Table 8. MBC of methanolic and aqueous extracts of *P. foetida* and *O. campechianum*

Dilutions	Concentrations (mg/mL)	*P. aeruginosa*		*K. pneumoniae*	
		Methanolic Extract	Aqueous Extract	Methanolic Extract	Aqueous Extract
P. foetida					
10^{-1}	150	-	+	+	+
10^{-2}	75	-	+	+	+
10^{-3}	38	+	+	+	+
10^{-4}	19	+	+	+	+
O. campechianum					
10^{-1}	150	+	+	+	+
10^{-2}	75	+	+	+	+
10^{-3}	38	+	+	+	+
10^{-4}	19	+	+	+	+

Note: (-) No growth; (+) Growth

Post Hoc Tukey test for *P. aeruginosa*

The methanolic *P. foetida* extract showed a significant difference between the aqueous *P. foetida* and *O. campechianum* extracts. The methanolic extract of *O. campechianum* showed a significant difference between the aqueous extracts of *P. foetida* and *O. campechianum*. In addition, the aqueous extract of *P. foetida* showed a significant difference from the aqueous *O. campechianum* extract (Table 9). No significant difference was observed between methanolic *P. foetida* and methanolic *O. campechianum* extract

In Table 9, methanolic *P. foetida* extract showed a significant difference between the aqueous *P. foetida* and *O. campechianum* extracts. The methanolic extract of *O. campechianum* showed a significant difference from the aqueous extracts of *P. foetida*. In addition, the aqueous extract of *P. foetida* showed a significant difference from the aqueous *O. campechianum* extract. No significance difference was observed between methanolic *P. foetida* and methanolic *O. campechianum* extract.

Post-Hoc Tukey test for *K. pneumoniae*

In Table 10, significance was observed between the aqueous *O. campechianum* extract and methanolic *P. foetida* extract; methanolic and aqueous *O. campechianum* extract, and aqueous *P. foetida* and *O. campechianum* extracts. In Table 10, significance existed between the aqueous *O. campechianum* extract with methanolic *P. foetida* extract; methanolic and aqueous *O. campechianum* extract, and aqueous *P. foetida* and *O. campechianum* extracts.

Table 9. Post Hoc statistical analysis of 0.15, 0.1, 0.05 concentration *P. foetida* and *O. campechianum* extracts

Tukey HSD	***P. foetida* and *O. campechianum* extract**		**Significance**
0.15 concentration	Methanolic extract of *P. foetida*	Methanolic extract of *O. campechianum*	1
		Aqueous extract of *P. foetida*	<.001
		Aqueous extract of *O. campechianum*	0.017
	Methanolic extract of *O. campechianum*	Aqueous extract of *P. foetida*	<.001
		Aqueous extract of *O. campechianum*	0.017
	Aqueous extract of *P. foetida*	Aqueous extract of *O. campechianum*	<.001
0.1 concentration	Methanolic extract of *P. foetida*	Methanolic extract of *O. campechianum*	0.085
		Aqueous extract of *P. foetida*	<.001
		Aqueous extract of *O. campechianum*	0.007
	Methanolic extract of *O. campechianum*	Aqueous extract of *P. foetida*	<.001
		Aqueous extract of *O. campechianum*	0.305
	Aqueous extract of *P. foetida*	Aqueous extract of *O. campechianum*	<.001
0.05 concentration	Methanolic extract of *P. foetida*	Methanolic extract of *O. campechianum*	0.414
		Aqueous extract of *P. foetida*	0.115
		Aqueous extract of *O. campechianum*	0.058
	Methanolic extract of *O. campechianum*	Aqueous extract of *P. foetida*	0.012
		Aqueous extract of *O. campechianum*	0.496
	Aqueous extract of *P. foetida*	Aqueous extract of *O. campechianum*	0.002

Table 10. Results of Post Hoc statistical analysis of 0.15 and 0.1 concentration *P. foetida* and *O. campechianum* extracts

Tukey HSD	***P. foetida* and *O. campechianum* extract**		**Significance**
0.15 concentration	Methanolic extract of *P. foetida*	Methanolic extract of *O. campechianum*	1
		Aqueous extract of *P. foetida*	1
		Aqueous extract of *O. campechianum*	<.001
	Methanolic extract of *O. campechianum*	Aqueous extract of *P. foetida*	1
		Aqueous extract of *O. campechianum*	<.001
	Aqueous extract of *P. foetida*	Aqueous extract of *O. campechianum*	<.001
0.1 concentration	Methanolic extract of *P. foetida*	Methanolic extract of *O. campechianum*	1
		Aqueous extract of *P. foetida*	1
		Aqueous extract of *O. campechianum*	<.001
	Methanolic extract of *O. campechianum*	Aqueous extract of *P. foetida*	1
		Aqueous extract of *O. campechianum*	<.001
	Aqueous extract of *P. foetida*	Aqueous extract of *O. campechianum*	<.001

Discussion

The use of medicinal plants to treat diseases is an old practice. It is the main source of care for approximately 85% of the world and the derivative for 80% of all synthetic drugs (Fitzgerald et al. 2020). The emergence of antimicrobial resistance has resulted in increased interest in these medicinal plants. They produce secondary metabolites which are not essential for their normal growth but aid in reproduction and act as defense mechanisms against bacteria, viruses, fungi, etc. Secondary metabolites have proven to be effective against both gram -ve and +ve bacteria, additionally, plant-derived drugs break down the cell wall and membranes of microbes, resulting in cell death (Anand et al. 2019).

In the experiment conducted above, extracts were made by soaking the leaves of both plants in hexane, methanol, and water. Results showed that only methanol and water produced yield, which may be due to the polarity of solvents. Hexane is a nonpolar solvent and as the plant may contain few or no non-polar molecules, no yield was produced. The methanolic extract of *O. campechianum* produced the highest percent yield (5.21%), followed by the aqueous *P. foetida* extract (3.94%), then the methanolic *P. foetida* (2.82%), and finally the aqueous *O. campechianum* extract (2.45%). The *O. campechianum* methanolic extract had the largest yield of all solvents. Although, yields of the extracts varied. This may be a result of spills when handling the dried extract and also spillage of concentrated liquid during filtration. According to Azwanida (2015), on the extraction methods of medicinal plants, a particle size smaller than 0.5 mm is ideal for efficient extraction. As such, the smaller the size of the leaves the better the extraction. The leaves used for maceration were small strips and as such the particle size may have also hindered extraction of the plant compounds.

The phytochemical analysis results of *P. foetida* and *O. campechianum* showed a wider range of compounds were present in *O. campechianum* than in *P. foetida*. Of the two solvents used, methanolic extracts were found to have more plant compounds. Flavonoids, tannins, glycosides, and phenols were found to be present in the methanolic extract of *O. campechianum,* while flavonoids, tannings, and

glycosides were found in the methanolic extract of *P. foetida*. The aqueous extracts of *O. campechianum* were found to contain tannins, glycosides, and saponins, while the aqueous extract of *P. foetida* only contained glycosides and saponins. The results of *P. foetida* are dissimilar to those in Birudu et al. (2015) and Prasanth et al. (2018) which displayed a wider range of compounds inclusive of flavonoids, tannins, and phenols in the aqueous extract. This difference may be because of the area in which the plants were grown. Secondary metabolites such as flavonoids combat resistance by inhibition of virulence factors, efflux pump, biofilm formation, membrane disruption, cell envelope synthesis, nucleic acid synthesis, and bacterial motility inhibition (Biharee et al. 2020). While phenols combat resistance excluding flavonoids, combat resistance by cell membrane disruption, inhibition of DNA gyrase, inhibition of helicase activity, multi-drug efflux pump inhibitors, dehydratase, and protein kinase inhibition (Rempe et al. 2017). The *O. campechianum,* unlike *P. foetida,* contains both flavonoids and phenols which may explain its greater antibiotic activity against the pathogens. In addition, the administration of flavonoids to *P. aeruginosa* alters transcription of quorum sensing-controlled target promoters and suppresses virulence factor production (Paczkowski et al. 2017). This confirms the effectiveness of both *O. campechianum* and *P. foetida* against *P. aeruginosa.*

In plants, the accumulation of secondary metabolites varies with a number of factors, such as light, temperature, soil water, soil fertility, and salinity (Yang et al. 2018). Any change in either of the factors may cause an alteration in the secondary metabolites present. Kumar et al. (2019) analyzed the effect of geographical and seasonal variations on the phenolic contents of antioxidant activity from different plant parts. It was found that altitude and seasonal variations significantly affect the secondary metabolites in plant parts. Research on *P. foetida* in Guyana is limited so further similarities could not be made. The present study showed that results of methanolic extract are similar to those obtained in a previous study on methanolic root extract of *P. foetida* by Emin et al. (2010). Research of *O. campechianum* is limited so no comparison to previous research was made.

The antimicrobial analysis unearthed that *O. campechianum* showed better antimicrobial activity than *P. foetida*, and *P. aeruginosa* isolate was more susceptible to both plants than *K. pneumoniae*. The aqueous extract proved to have greater antibiotic properties than methanolic extract of *O. campechianum* as larger zones were seen. The methanolic extract of *P. foetida* had more antibiotic content than aqueous extract. Zones of inhibition were only observed in the methanolic extract of *P. foetida* against *P. aeruginosa*.

The results obtained for *P. foetida* were unlike those seen in previous studies in different regions. A study done by Emin et al. (2010) revealed significant antibiotic activity of *P. foetida* against both *K. pneumoniae* and *P. aeruginosa*. The concentration of extract from 250-1250 mcg obtained zones of 7-13 mm and 14-22 mm for *K. pneumoniae* and *P. aeruginosa,* respectively. In contrast, no antimicrobial activity was observed against *K.pneumoniae*. Inhibition zones of 8 mm, 9 mm, and 11 mm from 50 mg/mL, 100 mg/mL, and 150 mg/mL concentrations respectively were observed for *P.aeruginosa*. No previous research with *O. campechianum* was found regarding the pathogens.

Ciprofloxacin, the positive control in this experiment, used a concentration of 0.1 g/mL and obtained areas above 30 mm for both pathogens. This is much different compared to the results obtained using the extracts of both plants. Results obtained for the two plants also displayed that there was a significant difference between the means of the extracts against *K. pneumoniae* and *P. aeruginosa*.

The MIC of *O. campechianum* against *P. aeruginosa* observed was 38 mg/mL and 19 mg/mL in methanolic and aqueous extracts respectively. While the MIC of *O. campechianum* against *K. pneumoniae* was 150 mg/mL for the aqueous extract. The MIC of *P. foetida* against *P. aeruginosa* was 38 mg/mL for the methanolic extract. The only plant which displayed bactericidal properties was *P. foetida* which had an MBC of 75 mg/mL against *P. aeruginosa*. This suggests that although *O. campechianum* is better at inhibiting bacterial growth of *P. aeruginosa* and *K. pneumoniae* than *P. foetida*, the plant cannot kill *P. aeruginosa,* unlike *P. foetida*.

Statistical analysis results of one-way ANOVA for *P. aeruginosa* revealed a significant difference between *P. foetida* and *O. campechianum* extracts. All concentrations (0.15 g/mL, 0.l g/mL and 0.05 g/mL) showed significance with P-values <0.001, <0.001 and 0.002. In contrast, statistical analysis of *K. pneumoniae* revealed a significant difference between *P. foetida* and *O. campechianum* extracts. Significance was seen at 0.15 mg/mL and 0.1 mg/mL with P-values <0.001 and <0.001. At 0.05mg/mL the P value was >0.05.

In conclusion, the phytochemicals present in *O. campechianum* were flavonoids, tannins, saponins, glycosides, and phenols, while flavonoids, tannins, glycosides, and saponins were found in *P. foetida* extract. The *O. campechianum* can inhibit but not kill both *P. aeruginosa* and *K. pneumoniae*, while *P. foetida* can kill and inhibit the growth of *P. aeruginosa*. The *P. foetida* displayed no antibiotic activity against *K. pneumoniae.* The *O. campechianum* was statistically significant compared to *P. foetida* against *K. pneumoniae*, whereas *P. foetida* was statistically significant compared to *O. campechianum* against *P. aeruginosa*. The *O. campechianum* can be used to inhibit and control the growth of both pathogens.

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