

## Full Length Research Paper

## The antibacterial and antifungal analysis of crude extracts from the leaves and bark of *Pimenta* species found in Jamaica

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Due to the rapid increase in the number of antibiotic-resistant bacteria, medicinal plants are being studied as new and promising alternatives to conventional antibiotic treatment. The crude hexane, ethyl acetate and methanol extracts from *Pimenta dioica*, *Pimenta jamaicensis* and *Pimenta racemosa* were quantitatively assessed to determine their antimicrobial susceptibility and potency using zones of inhibition methods, minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) or fungicidal concentrations (MFC) against *Streptococcus A*, *Streptococcus B*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella* species, *Proteus mirabilis* and *Candida albicans*. The crude ethyl acetate extract of the *P. dioica* was most active against *Candida albicans* (MFC of 1.3 mg/mL and MIC of 0.63 mg/mL) while the crude hexane extract of *P. jamaicensis* was most active against *Streptococcus A* (MBC of 0.63 and MIC of 1.3 mg/mL). The crude ethyl acetate extract of *P. racemosa* was most active against *Streptococcus A* and *Salmonella* (MBC of 2.5 mg/mL and MIC of 1.3 mg/mL; MBC of 0.63 and MIC of 0.63 mg/mL, respectively). Extracts from selected species of *Pimenta* may potentially provide a source of new antimicrobial agents for the treatment of infectious diseases.

**Key words:** *Pimenta dioica*, *Pimenta jamaicensis*, *Pimenta racemosa*, antimicrobial.

### INTRODUCTION

The spice of Jamaican cuisine is often times completed with a dash of *Pimenta*, also known as allspice, Jamaican

pepper, new spice or myrtle pepper. Its distinct flavor results from its unique blend of terpenes and other

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secondary metabolites. These compounds have also contributed to the medicinal properties of the plant and therefore used to treat various ailments in Jamaican folk medicine. The genus *Pimenta* contains about 14 species from the Myrtaceae family, all of which are indigenous to the Caribbean and Central America; Jamaica has 6 endemic species found within the genus (Adams, 1976). The species that may be found in Jamaica include *Pimenta dioica* (Allspice), *Pimenta racemosa* (bay rum), *Pimenta jamaicensis* (wild pimento), *Pimenta obscura*, *Pimenta richardii* and a species not yet identified (Institute of Jamaica Identification number: P22536B). These flowering plants may be monoecious or dioecious and contain many active ingredients. *Pimenta* is entrenched in many folklore remedies and used to treat cancer, diabetes, malaria and other chronic and infectious diseases.

The chemical profile of the *Pimenta* genus is distinct and contributes to the diversity in the use of the plant. The active components found in *P. dioica*, *P. obscura* and *P. richardii* are reported to be methyl eugenol, caryophyllene, and limonene identified via chemical analysis (Zhang et al., 2012). The medicinal properties for *P. dioica* and *P. racemosa* have been studied extensively. The essential oils and extracts from these plant species have been found to contain antimicrobial and antifungal properties (Martinelli et al., 2017; Hammer et al., 1999; Zabka et al., 2009), anti-nociceptive properties as well as anti-cancer activity against prostate cancer cell lines (Garcia et al., 2004). *P. dioica* also has significant cytotoxic, anti-oxidant, analgesic and anti-hypertensive effects (Suárez et al., 1997; Marzouk et al., 2007). The aqueous extract of the berries showed significant antitumor activity, while ethanolic extracts from the leaves and berries can also be used as a food preservative (Sessou et al., 2012). The macerated leaves of *P. dioica* have previously shown some antimicrobial activity with crude hexane and alcoholic extracts against *Pseudomonas fluorescens*, *Bacillus megaterium*, *Aspergillus niger* and *Penicillium* species (Boyd et al., 2014). Other studies have shown that essential oils obtained from *P. dioica* have the potential of being used as a natural pesticide and fungicide (Seo et al., 2009).

Essential oils of *P. racemosa* possess several types of bioactivity that have been validated experimentally. Alitonou et al. (2012) reported that the oil has low anti-inflammatory activity based on the lipoxygenase test results, as well as, antiradical, acaricidal and antimicrobial activity against both bacteria and fungi. It was postulated that the oils can be exploited industrially for its antioxidant activity to delay the degradation of fatty substances. Neither of the crude extracts (hexane, ethyl acetate and chloroform) of *P. racemosa* was found to have any significant effect as a cytotoxic agent. In a research conducted to assess the antimicrobial activity of 52 plants, *P. racemosa* was one of the three plants that showed anti-microbial activities on all the following

organisms: *Acinetobacter baumannii*, *Aeromonas veronii* biogroup *sobria*, *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enterica* subsp. *enterica* serotype *typhimurium*, *Serratia marcescens* and *Staphylococcus aureus*, using an agar dilution method (Hammer et al., 1999), which also formed a part of the basis for this study. Nesrin et al. (2017) have demonstrated that the most abundant component of essential oil obtained from *P. racemosa* is methyleugenol and suggested that the essential oil holds great promise as natural preservative and antimicrobial agents. *P. jamaicensis* contains eugenol and limonene which contributes to its characteristic smell and properties; other compounds include p-cymene, 1, 8-cineole,  $\beta$ -caryophyllene, and 4-terpineol as the main constituents (Tucker et al., 1992).

The potential for new antimicrobial agents is dependent on their experimental validation when extracts or compounds are exposed to different microorganisms. Plant-based secondary metabolites have immense potential for combating a broad spectrum antibiotic-resistant bacterium with little or no side effects (Chandra et al., 2017). The literature suggests that *Pimenta* species could hold significant promise as an antibiotic agent. In this study, the antifungal and antimicrobial activity of various crude extracts (hexane, methanol and ethyl acetate) was evaluated for *P. dioica*, *P. racemosa* and *P. jamaicensis*.

## MATERIALS AND METHODS

### Plant collection and extraction

The plant materials were obtained and verified by the taxonomist at The University of the West Indies, Mona Herbarium and samples were given reference numbers 35922, 35923 and 35924 for *P. dioica*, *P. racemosa*, and *P. jamaicensis*, respectively. The samples (leaves and bark) were dried at room temperature and milled into a powder prior to solvent extraction with hexane, ethyl acetate and methanol to obtain crude extracts of each species. The first solvent, hexane, was percolated through the plant material for 24 h, filtered, concentrated in vacuum under reduced pressure using rotary evaporator and dried in a desiccator to obtain the crude hexane extract. The same plant material was then treated with ethyl acetate and methanol sequentially for the same time period to obtain the crude ethyl acetate and methanol extracts. A sample of 0.1 g of the plant extracts was dissolved in 10 ml of dimethyl sulphoxide (DMSO) and a serial dilution was carried out to obtain 6 extract concentrations (10,000, 5000, 2500, 1250, 625, and 312.5  $\mu\text{g/mL}$ ) which was then used in the microbial test.

### Bacterial and fungal isolates

Bacterial isolates (Gram-positive: *Streptococcus* group A (ATCC 12386), *Streptococcus* group B (ATCC 19613), *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 25923) and Gram-negative: *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC BAA 1705), *Salmonella* species (5567 Nov 2011), *Proteus mirabilis* (7002) and fungal isolate (*C. albicans*; ATCC 14053)) used

for this study were obtained from The University of the West Indies, Mona (Microbiology Department).

### Anti-microbial assays

All the extracts were screened to assess their antibacterial and antifungal properties using agar-well diffusion method (Perez et al., 1990). The samples were further tested at 1 mg/mL (the maximum concentration at which an extract may be considered active controls) (DeMarsh et al., 2001). The minimal inhibitory concentration (MIC) of the active samples was determined using extracts obtained from leaves and bark of the respective *Pimenta* spp.

### Well diffusion assay

Suspensions made from 24 h bacterial or fungal cultures were standardized using 0.5 McFarland Standard (ca.  $10^8$  CFU/mL) and inoculated on the surface of Mueller-Hinton agar (bacteria) and Sabouraud dextrose agar (fungi) plates using sterile cotton swab (Baker et al., 1983). The diluted crude extract and the control (100  $\mu$ l of each) were then introduced into the wells of 6 mm diameter that were created in the agar. The plates were kept at 4°C for 1 h to permit diffusion of the extracts, then incubated at 37°C for 24 (bacteria) and 48 h (fungi) and the diameter of the zone of growth inhibition was then measured using a caliper. The experiment was repeated for reliability.

### Tube dilution assay

The extracts that showed antimicrobial activity were further tested to determine the MIC for each bacterial or fungicidal sample. 1 mL of each extract was added to 1 mL of sterile brain heart infusion broth and used to prepare two-fold broth micro-dilutions of the various extracts (Ferraro, 2000). The tubes were inoculated with a drop of microbial suspension and incubated for at 37°C for 24 h, after which the MICs were measured macroscopically to assess the efficacy and effectiveness of the extracts in comparison with the sterility and growth controls which were included for each assay (DeMarsh et al., 2001). All the tubes were evaluated for growth indicated by turbidity. The samples at each concentration, sterile and growth controls were then seeded onto blood agar plates and incubated at 37°C for 24 h after which the minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) was recorded. The MBC or MFC was defined as the lowest concentration of the extract associated with no bacterial or fungal growth on a freshly inoculated agar plate.

## RESULTS

The antibacterial and antifungal activity of *P. jamaicensis*, *P. dioica* and *P. racemosa* are outlined (Tables 1 and 2). All the leaf extracts of the investigated *Pimenta* spp. showed significant antimicrobial activity against one or more microbes, except for the crude hexane extract of *P. dioica* and crude methanol extract of *P. racemosa* (Table 1). *P. racemosa* was the most active of the three *Pimenta* spp., with the ethyl acetate extract being most active against *Salmonella* and *Streptococcus B* and the hexane extract being most active against *C. albicans*. The ethyl acetate extract of *P. dioica* and hexane extract of *P.*

*jamaicensis* were most active against *C. albicans* and *Streptococcus A*, respectively. There was no significant antimicrobial activity observed for *E. faecalis*, *E. coli*, *P. mirabilis* and *K. pneumonia* using any of the extracts.

The MBCs and MICs exhibited by the extract against test bacteria ranged between 0.63 and 2.5 mg/mL and 0.63 and 1.3 mg/mL, respectively (Table 2). The growth of *C. albicans* was inhibited by *P. dioica* leaf ethyl acetate extracts giving a MFC of 1.3 mg/mL and MIC of 0.63 mg/mL. Of the three plant species, the *P. racemosa* leaf ethyl acetate extracts were the most potent antibacterial agents against the Gram-positive bacteria, *Streptococcus B* (MBC of 0.63 mg/mL and MIC of 0.63 mg/mL). This extract also showed an antibacterial effect against the Gram-negative bacteria, *Salmonella* spp., but was less potent (MBC of 2.5 mg/mL and MIC of 1.3 mg/mL).

## DISCUSSION

*Pimenta* spp. have shown significant promise based on the preliminary tubal broth dilution assays and subsequent inhibitory and bactericidal tests conducted. The MIC indicates the lowest concentration that can inhibit growth, while the MBC determines the concentration at which a compound or extract will kill the bacteria being tested. The MIC and MBC are complementary in that the extract or compound would be most effective as the MIC determines if it is anti-proliferative and then the MBC determines if it is also bactericidal.

The results reveal that both Gram-positive and Gram-negative bacteria and the fungi *C. albicans* were susceptible to the effects of the extracts obtained from *Pimenta* plant species. As outlined in Table 1, all three crude leaves extracts of the endemic *P. jamaicensis* showed antibacterial activities against microbes such as Streptococci which are known to cause numerous ailments. *Streptococcus A* (Group A Streptococcus, GAS) was susceptible to the effect of the *P. jamaicensis* crude extracts, however, the hexane extract was the most active, while the crude ethyl acetate and methanol extracts were partially active against *Streptococcus B*. *Streptococcus A* is usually spread via mucus from the nose, throat or via skin, and is often treated with antibiotics. Over 500,000 deaths have been reported per year for GAS related diseases (Cohen-Poradosu and Kasper, 2007). The *P. jamaicensis* leaf hexane extract could be an effective natural astringent or disinfectant which could aid with growth control of *Streptococcus A*. *Streptococcus B* or *Streptococcus agalactiae* (Group B Streptococcus, GBS) are bacteria also found commonly in the human body that may cause infections, especially with pregnant women and newborns. The highest cases of GBS infections were observed in non-pregnant women above 65 years old, with the incidence decreasing exponentially with age. The *P. racemosa* ethyl acetate

**Table 1.** Antibacterial and antifungal activity of *Pimenta dioica*, *Pimenta jamaicensis* and *Pimenta racemosa* leaf extracts at 1 mg/mL through well diffusion method.

Plant specimen	Diameter of the zone of inhibition (mm) in 1 mg/mL		
	Methanol	Ethyl acetate	Hexane
<i>Pimenta jamaicensis</i>	<i>S. aureus</i> (9)	<i>Streptococcus</i> A (10),	<i>Streptococcus</i> A (15)
	<i>Streptococcus</i> A (11)	<i>Streptococcus</i> B (10),	-
	<i>Streptococcus</i> B (12)	<i>P. aeruginosa</i> (10)	-
<i>Pimenta dioica</i>	<i>Salmonella</i> (10)	<i>Candida albicans</i> (14)	NA
	<i>Streptococcus</i> A (11)	<i>P. aeruginosa</i> (9)	-
	<i>Streptococcus</i> B (9)	-	-
<i>Pimenta racemosa</i>	NA	<i>Salmonella</i> (16),	<i>Candida albicans</i> (15)
	-	<i>Streptococcus</i> B (13)	<i>Streptococcus</i> B (9)
	-	-	<i>P. aeruginosa</i> (10)

6-8, Not active (NA); 9-12, partially active; 13-17, active; >17, very active.  
Source: DeMarsh et al. (2001).

**Table 2.** The MBCs/MFCs and MICs of the most effective crude extract of *P. jamaicensis*, *P. dioica* and *P. racemosa* using tube dilution assay.

Crude extract	Organism	MBC/MFC (%)	MIC (%)
<i>Pimenta dioica</i> leaf ethyl acetate	<i>Candida albicans</i>	1.3 mg/mL (100)	0.63 mg/mL (99)
<i>Pimenta racemosa</i> leaf ethyl acetate	<i>Salmonella</i> spp.	2.5 mg/mL (100)	1.3 mg/mL (50)
	<i>Streptococcus</i> B	0.63 mg/mL (100)	0.63 mg/mL (100)
<i>Pimenta jamaicensis</i> leaf hexane	<i>Streptococcus</i> A	1.3 mg/mL (100)	0.63 mg/mL (90)

extract was active against *Streptococcus* B with MBC of 0.63 mg/mL and MIC of 0.63 mg/mL which is comparable to that of fluconazole (Barchiesi et al., 1994), while the hexane extract showed partial activity. The *P. jamaicensis* ethyl acetate leaf and methanol extracts and *P. dioica* methanol leaf extract also showed partial activity for GBS.

All three *Pimenta* species crude leaves extracts were partially active against *P. aeruginosa*, which has multiple-mechanisms of resistance to antibiotics and can only be treated by a selected number of anti-pseudomonal agents. This gram-negative bacterium has about 13 different strains, which is often implicated in ailments such as pneumonia, urinary tract infections, gastrointestinal infections, hemorrhage, and nosocomial infections. It thrives on moisture and as such is often found in persons hospitalized for over a week via cross infection due to catheters and other medical equipment. Its presence inside the body can be fatal when associated with some of the major organs such as the kidney and lungs. *P. aeruginosa* growth may be reduced or retarded by the *P. racemosa* hexane leaf extract, *P.*

*dioica* ethyl acetate leaf extract, and the *P. jamaicensis* ethyl acetate leaf extract.

Interestingly, the gram-negative *Salmonella* spp. which is often associated with gastrointestinal diseases, are susceptible to the effect of *P. dioica* and *P. racemosa* which are often added to food to enhance the flavor. The crude methanol extract of *P. dioica* was partially active while the crude ethyl acetate *P. racemosa* was active against *Salmonella* spp. This bacteria has been implicated in over one million cases of food poisoning annually (Centre for Disease Control and Prevention, 2016). According to the Centre for Disease Control and Prevention, *Salmonella* infection can be extreme and cause death, as reported in 2013 where over 19,000 patients were hospitalized for salmonellosis, from which 378 died. These infections are preventable with simple hands hygiene techniques, the separating of uncooked and cooked foods, cooking food properly and keeping foods refrigerated. Nonetheless, these crude extracts may have applications as an antimicrobial agent, to reduce *Salmonella* growth and as such reduce the prevalence of food borne illnesses.

*C. albicans* is a yeast-like fungus that forms a part of the normal intestinal microbiota of 70% of the human population. Its overgrowth may cause infections when there is favorable change in the internal conditions of the body, which results in candidiasis making this fungus the fourth leading cause of nosocomial infection (Pfaller and Diekema, 2007). Infections involving skin or mucosal surfaces are usually combated with oral or topical agents. *C. albicans* may, however, become invasive, traveling throughout the bloodstream causing damage to major organs requiring parenteral antifungal therapy. The *P. racemosa* and *P. dioica* were the most effective against *C. albicans* with the crude hexane leaf extract and the crude ethyl acetate leaf extract, respectively showing significant activity in the initial screening of the microbes. In the second stage of antimicrobial confirmation, the MICs of the *P. jamaicensis* ethyl acetate crude leaf extract was 99% (0.63 mg/mL) against *C. albicans* which was comparable to fluconazole, an antifungal, which usually has MIC of 92% against this organism (Barchiesi et al., 1994).

## Conclusion

The use of *Pimenta* spp. has scientific credential as an antimicrobial agent. This is particularly important as globally there has been an increase in antimicrobial resistance, while fewer antimicrobial agents are being made, creating a possible scenario where there are no options for treatment. Most significant activity for *P. jamaicensis* was against the *Streptococcus A* bacteria, *P. dioica* was active against *C. albicans* and *P. racemosa* was active against *Salmonella*, *Streptococcus B* and *C. albicans*. These *Pimenta* species showed significant inhibitory and antimicrobial properties against the respective microbes and may be further studied in order to see whether they can be effectively incorporated into disinfecting, antimicrobial or antiseptic formulations to aid in combating diseases caused by these microbes.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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