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Phytochemical screening and *in vitro* antioxidant and antimicrobial activities of the extracts of the stem-bark of *Psydrax peruviana*

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Abstract
The stem-bark of *Psydrax peruviana* is used in folkloric medicine in the treatment of gonorrhoea and syphilis. In this study, methanol and hexane extracts of *P. peruviana* were evaluated for antimicrobial and antioxidant properties. Phytochemical screening, antimicrobial (agar and broth dilution method) and antioxidant (total antioxidant capacity (TAC), DPPH and H_2O_2 scavenging) assays were carried out on the extracts. The hexane and methanol extracts showed antimicrobial activity against test organisms with MICs ranging from 1.25 – 20 mg/mL. The IC_{50} values for methanol and hexane extracts in the DPPH and H_2O_2 assays were 12.20 and 24.26 µg/mL and 1143.0 and 2872.0 µg/mL, respectively. The TAC (gAAE/100 g) for methanol and hexane extracts were 21.563 and 17.389 g, respectively. The phytochemical investigation revealed the presence of plant secondary metabolites such as tannins, flavonoid, glycosides, terpenoids, steroids, carotenoids and saponins. These findings provide scientific justification for the use of *P. peruviana* in various traditional medicines, for the treatment and management of diseases.

Keywords: *Psydrax peruviana*, antimicrobial, antioxidant, phytochemical, assay, *in-vitro*

1. Introduction
Medicinal plants and human beings have special relationship for centuries. Man's interest in plants, primarily as a source of food, shelter and clothing, dates back to the beginning of human progression [1]. Medicinal plants are acknowledged as herbal medicines and are used all over the world from primal time [2]. Traditional medicines have been used to cure, treat, prevent and diagnose ailments for centuries [3]. Many plants have been identified to exhibit antimicrobial, antioxidant and anti-inflammatory activities [4-5]. A considerable number of currently used antioxidant, anti-infectious, anthelminthic, anti-inflammatory, antitumor agents are molecules identified and isolated from plants or their synthetic or semisynthetic derivatives [6]. The action of these medicines is attributed to the active components present in the various parts of plant [7].

Psydrax is a genus found throughout the Old World tropics from Africa, throughout southern and South East (SE) Asia, to Australia [8]. *Psydrax* is a genus of flowering plants in the *Rubiaceae* family which consists of trees, shrubs and a few lianas in the paleotropics. Joseph Gaertner in 1788 named the genus in his book De Fructibus et Seminibus Plantarum [9]. *Psydrax* is a Greek word meaning a blister or bump. The warty fruit or the pimply seeds of some species might have led to the name chosen by Gaertner [10]. After Gaertner proposed the name, most authors hardly used the name and also placed the species under *Canthium*. In 1985 *Psydrax* was reinstated and 37 African species were transferred to it from *Canthium* [11]. *Psydrax* is closely related to Afrocanthium, Cyclophyllum and Keetia, genera that have been separated from *Canthium* [12]. This plant is locally known among the Ashanti tribe in Ghana as 'Ananse Ponua'. Herbal medicine practitioners in Ghana, use this plant to treat degenerative and mostly sexually transmitted infections due to its antimicrobial properties. The decoction of the stem-bark and that of the leaves of this plant is taken orally to alleviate diseases such as gonorrhoea and syphilis.

Antimicrobials can be used as therapy for bacteria (antibacterial), viruses (antiviral), fungi (antifungal) or protozoa (antiprotozoal). Sulphonamide was the first antimicrobial to be introduced to food animal medicine in the 1940s [13].

Resistance by microbes to antibiotics is inevitable, therefore some bioactive and efficacious antibiotics are becoming less effective due to the development of resistance by microbes. The global crisis of microbial resistance has threatened the effective treatment and prevention of an ever-increasing range of infections caused by microbes [14]. Modern medicines, which are based on synthetic drugs and antibiotics, mostly relied on herbal medicine but many pharmacological active medicinal plants claimed by the local medical practitioners have not been scientifically tested and *Psydrax peruviana* plant are included.

Excessive amount of reactive oxygen and nitrogen species (RONS) may be harmful because they can initiate bimolecular oxidation which leads to cell injury and death, oxidative stress which result in numerous diseases and disorders such as-aging, cancer, atherosclerosis, cirrhosis and Alzheimer [15]. Antioxidants ability to scavenge and inhibit (RONS), results in protection from oxidative harm and are accordingly viewed as essential therapeutic and prophylactic agents against the development of diseases [16]. However, some synthetic variants may pose a threat to humans due to their associated adverse effects on living cells. Thus, the interest in utilizing and screening sources for novel natural antioxidants from natural products is an urgent need.

At the time of carrying out this research, next to nothing had been carried out on the stem-bark of the *P. peruviana* plant. The aim of this study was to examine the efficacy of *P. peruviana* methanol and hexane extracts of the stem bark as an antimicrobial and antioxidant agent using *in vitro* assays.

2. Materials and Methods

2.1 Sample collection and identification

The stem-bark of *Psydrax peruviana* were collected in the month of October, 2018 at Adansi-Asokwa in the northern part of Adansi in the Ashanti Region of Ghana with the help of a local herbalist. They were taxonomically identified and authenticated by Mr. Clifford Asare at the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, KNUST, Kumasi, Ghana. A voucher specimen number (KNUST/HMI/2018/L014) was deposited in the Herbarium of Faculty of Pharmacy and Herbal Medicine for reference purposes.

2.2 Chemicals and reagents

All chemicals were purchased from Sigma Aldrich Co. Ltd, Irvine, U.K., except the standard drugs. The organic solvents were of analytical grade and procured from BDH Laboratory Supplies, U.K.

2.3 Extraction of plant material

The stem-barks of *P. peruviana* were thoroughly washed first under running water and then distilled water. The stem-barks were chopped into smaller pieces, air dried under shade for two weeks, pulverized into coarse powder, and stored in a desiccator until analysis.

2.3.1 Preparation of methanol extract

Maceration was used for the extraction of the polar constituents of the pulverized sample. A mass of 200 g of the pulverized sample of *P. peruviana* was soaked in 750 mL of methanol and macerated with gentle stirring for 72 hours at ambient temperature. The extract was condensed and evaporated to dryness using the rotary evaporator at 50 ºC (BUCHI Rota vapor R -114). The extract was dried and the percentage-yield of extract with respect to powdered plant material was determined. The methanol extract was then stored at 4 ºC in a refrigerator.

2.3.2 Preparation of hexane extract

A mass of 243 g of the powdered sample of *P. peruviana* was soaked in 750 mL of *n*-hexane and extracted using the soxhlet apparatus. The extract was condensed and evaporated to dryness using the rotary evaporator at 50 ºC (BUCHI Rota vapor R -114). The extract was dried and the percentage yield of extracts with respect to powdered plant material was determined. The hexane extract was then stored at 4 ºC in a refrigerator.

2.4 Phytochemical screening of extracts

The pulverized sample and the crude extracts obtained were screened to assess the presence of phytoconstituents using the methods described by Trease and Evans (2009) [17].

2.5 *In vitro* antioxidant assays

Three main assays were employed for the antioxidant activity determination. They were the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals scavenging, Hydrogen Peroxide scavenging (H_2O_2) and the Total Antioxidant Capacity (TAC) assays.

2.5.1 DPPH radical scavenging assay

The DPPH free radical scavenging activity of the three extracts were examined according to a modification of the standard methods previously described [5, 18]. Ascorbic acid was used as standard reference drug. The absorbance was measured at 517 nm. DPPH radical scavenging (%) was calculated using the formula:

$$\%\ Scavenging = \frac{A_o - A}{A_o} \times 100\%$$

Where, A_0 = absorbance of control; A = absorbance of test solution

2.5.2 Hydrogen peroxide scavenging assay

Determination of hydrogen peroxide scavenging potential of the extracts were carried out according to a modification of-the standard methods previously described [5, 19]. Gallic acid was used as standard reference drug. Absorbance was taken at 510 nm using a UV-vis spectrophotometer. The percentage scavenging activity was calculated using the formula below

$$\%\ Scavenging = \frac{A_{test}}{A_{control}} \times 100\%$$

Where A_{test} absorbance of the test samples and $A_{control}$ is the absorbance of the negative control. The results were further reported in IC_{50}.

2.5.3 Total antioxidant capacity (TAC) assay

A modification of the methodology as previously described was used to study the total antioxidant capacity of the extracts of *P. peruviana* [5, 20]. Ascorbic acid was used as the standard reference antioxidant drug. The absorbance of the solutions was measured in triplicates using a UV-visible spectrophotometer at 695 nm. The absorbance was measured and distilled water was used as the blank. From the linear equation of the ascorbic acid concentration-absorbance plot, the corresponding independent variables as ascorbic acid equivalents (AAE) were determined, and the results expressed

as gAAE/100g ascorbic acid.

2.6 Antimicrobial activity
Agar well diffusion and Broth micro-dilution (minimum inhibitory concentration) assays were employed to assess the antimicrobial activities of the extracts.

2.6.1 Sources of microorganisms
Four bacteria and one fungus were used as test organisms. These were two Gram positive bacteria which included *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram negative bacteria which included *Escherichia coli*, *Pseudomonas aeruginosa*. The fungus was *Candida albicans*. The microbial strains were provided by the Pharmaceutical Microbiology Section of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Science, KNUST, Kumasi. The microbial strains were sub-cultured on nutrient agar slants and incubated at 37 °C for 24 hours.

2.6.2 Inoculum preparation: Bacterial isolates were streaked onto nutrient agar (Oxoid, United Kingdom) plates and incubated for 18–24 hours at 37 °C. Using the direct colony suspension method, suspensions of the organisms were made in nutrient broth and incubated overnight at 37 °C. For the tests, colony suspensions in sterile saline was adjusted to 0.5 McFarland standard and further diluted in sterile double strength nutrient broth (~2×10^5 CFU/mL) [21].

2.6.3 Agar well Diffusion
The antimicrobial activities of the different extracts were determined using the agar well diffusion method as described by Agyare *et al*., 2004 [22]. In this method, the inoculum of bacteria was prepared from 24 hour broth cultures and inoculum of candida from a 48-hour broth culture and serial dilutions were made to achieve a suspension of ~2.0×10^5 CFU/mL. One milliliter of the diluted cultures was inoculated into sterile molten agar at 45 °C and poured into a sterile petri dish. They were swirled gently and allowed to solidify. In each petri dish, 6 wells equidistant from each other were bored into the solidified inoculated nutrient agar plates using cork borer number 5. Different concentrations of 20 and 10 mg/mL of the extracts, ciprofloxacin (0.05 mg/mL), clotrimazole (0.05 mg/mL) and control were introduced into the wells after which the plates were incubated at 37 °C for 24 hours. At the end of the incubation period, the diameter of the inhibition zone(s) was measured and recorded. The extracts and antibiotics were tested in triplicates and mean zones of inhibition were calculated for each extract and the standard antibiotic.

2.6.4 Broth micro-dilution
In the determination of the minimum inhibitory concentration (MIC), the method used was micro-well broth dilution described by Agyare *et al.*, 2004 [22] with slight modification. The inoculum of microorganisms was prepared from 24-hour broth cultures and serial dilutions were made to achieve a suspension of approximately 2.0×10^5 CFU/mL. The 96-well sterile plates were prepared by dispensing into each well 100 µL of double strength nutrient broth and calculated volumes of the stock solutions of extracts were added to the appropriately labeled wells to achieve concentrations of 20, 10, 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL. Twenty microliters of the freshly prepared inoculum was then transferred into each well to obtain a final volume of 200 µL. The micro plates were incubated at 37 °C for 24 hours. Growth of the microorganisms was determined by adding 20 µL of a solution of tetrazolium salt (3-(4, 5 dimethylimidazole-2yl-2,5-diphenyltetrazolium bromide) (MTT) and incubating for a further 30 minutes. Dark purple colouration of wells indicated the presence of viable microorganisms whiles no colour change indicated the absence of viable cells. The MIC was determined as the least concentration which showed no colour change after addition of MTT. Ciprofloxacin and clotrimazole were used as positive control. The experiment was carried out in triplicate.

2.7. Thin Layer Chromatography (TLC)
The number of components present in the extracts were determined by the analytical TLC method. The pre-coated silica gel plates (0.25 mm) with a fluorescent indicator (F254) were spotted with the extracts about 1 cm from the bottom edge of plates, with the aid of capillary tubes and allowed to dry [23]. Various solvent systems of petroleum ether/ethyl acetate and hexane/ethyl acetate in the ratio of 9:1 and 8:2 respectively were used. The ratio of 8:2 (hexane/ethyl acetate) gave the best separation of most of the components for all the extracts. The plates were dried and visualized by a 254 nm UV lamp. The separated spots were then marked and their sample and solvent fronts were measured.
The retardation factor (R_f) of the eluted spots was calculated as follows:

$$R_f = \frac{\text{Distance travelled by spot}}{\text{Distance travelled by solvent front}}$$

2.8 Data Analysis
Microsoft Excel 2016 and GraphPad Prism 6.0 for Windows (GraphPad Sofware, San Diego, CA, USA) were used for all data analyses and graphs.

3. Results and Discussion
3.1 Extraction of plant material
The yields of the extract in relation to the powdered plant material were calculated as percentages. The yields were 9.80% w/w and 2.65% w/w for methanol and hexane extracts, respectively.

3.2 Phytochemical Screening
The therapeutic activities of plants are as a result of the presence of complex chemical constituents in different parts [24]. The phytochemical screening revealed the presence of seven secondary metabolites out of the nine tested for in the pulverized sample and the methanol extract with the exception of alkaloids and anthraquinones. Alkaloids, anthraquinones and saponins were absent in the hexane extract (Table 1).

Table 1: Phytochemical constituents of the pulverized sample and the extracts of *P. peruviana*

Phytochemical	Pulverized sample	Methanol extract	Hexane extract
Saponins	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+
Flavonoids	+	+	+
Alkaloids	-	-	-
Glycosides	+	+	+
Anthraquinones	-	-	-
Carotenoids	+	+	+

Key: (+) = presence of secondary metabolite; (-) = absence of secondary metabolite

The methanol and hexane extracts had six phytochemicals in common, that is tannins, terpenoids, steroids, flavonoids, glycosides and carotenoids. The absence of saponins (which are polar due to their hydrophilic glycoside moieties) in the hexane extract might be due to the fact that the solvent is more non polar hence could not extract the saponins from the powdered plant material. Methanol extract, however, showed the presence of saponins due to its polarity. Secondary metabolites of plants which include flavonoids, anthocyanins, tannins, terpenoids, and phenolics acids have been shown to exhibit various pharmacological activities such as antioxidant, antispasmodic, antifungal and antimicrobial [25]. Saponins have a wide range of pharmacological properties, including antifungal, antiparasitic, molluscicidal and anti-inflammatory [26]. Flavonoids and its derivatives have many interesting pharmacological activities including antitumor, antioxidant, anti-inflammatory, antidiabetic and enzyme inhibitory activities [27, 28]. The presence of these phytochemicals in the extracts of *P. peruviana* stem-bark indicate that they will play a key role in the prevention of various bacterial infections and oxidative-stress diseases.

3.3 *In Vitro* Antioxidant Capacity

The total antioxidant potential of a plant extract depends largely on both the constituent of the extract and the test system [5]. Different factors can also influence the activity of the extract, and therefore antioxidant capacity cannot be fully determined and understood by one single method [29]. Considering the various mechanisms of antioxidant actions, the anti-oxidant properties of the extracts were evaluated by DPPH free radicals scavenging, Hydrogen Peroxide scavenging and the Total Antioxidant Capacity assays.

3.3.1 DPPH radical scavenging capacity

The DPPH radical scavenging activity of the extracts was used to determine and study the ability of the extracts of *P. peruviana* to mop up free radicals that may be found in animals and humans. Methanol and hexane extracts of *P. peruviana* and ascorbic acid (standard drug) scavenged DPPH radical in a dose dependent manner (Figure 1).

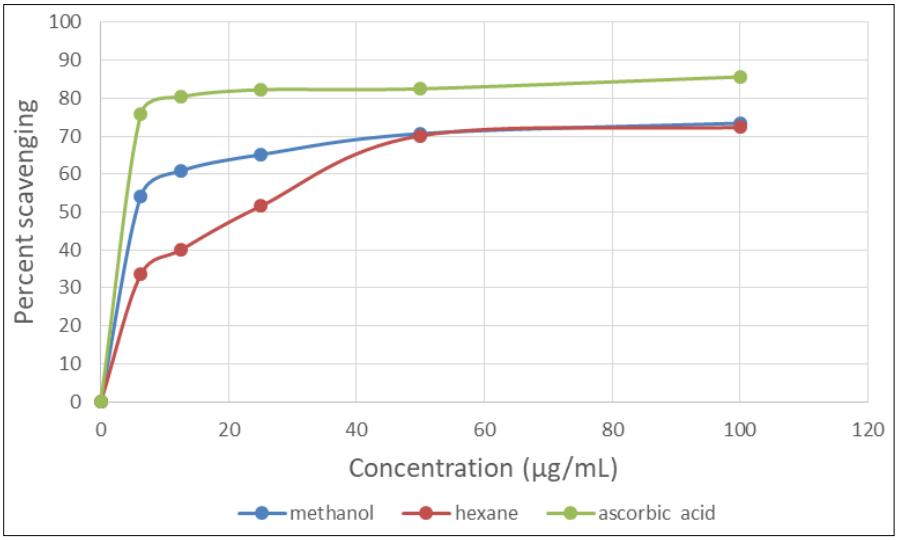

Fig 1: Comparative radical scavenging activity of the hexane and methanol extracts and ascorbic acid.

The reference antioxidant (ascorbic acid), hexane and methanol extracts of *P. peruviana* showed antioxidant activity in the DPPH free radical scavenging assay with IC_{50} of ascorbic acid, hexane and methanol ranging between 2.92 and 24.26 µg/mL as shown in Table 2.

Table 2: IC_{50} of DPPH Radical scavenging activity for hexane and methanol extracts and ascorbic acid

Sample	IC_{50} (µg/mL)
Ascorbic acid	2.92
Methanol	12.20
Hexane	24.26

The results implied that the potency of the test samples of extracts as antioxidants decreased in the order: ascorbic acid > methanol > hexane (Figure 1). Though hexane and methanol extracts which comprise of a mixture of compounds were not as potent as the ascorbic acid, *P. peruviana* stem-bark extracts may be useful in the manufacture of drugs to help prevent or cure health problems that could arise from the systemic actions of oxidative agents.

3.3.2 Hydrogen peroxide scavenging assay

Non-radical oxidizing agents scavenging potential of the hexane and methanol extracts of *P. peruviana* were evaluated by the use of hydrogen peroxide (H_2O_2) scavenging method. The results are shown in Table 3.

Table 3: Hydrogen Peroxide scavenging capacity of Hexane and Methanol extracts and Gallic acid.

Concentration (µg/mL)	% Inhibition (Mean ± SD)		
	Methanol	Hexane	Gallic acid
1000	60.67 ± 0.018	69.04 ± 0.014	57.89 ± 0.002
800	54.64 ± 0.002	64.86 ± 0.016	53.74 ± 0.020
600	41.11 ± 0.022	47.11 ± 0.018	54.84 ± 0.006
400	36.13 ± 0.017	43.83 ± 0.019	55.60 ± 0.012
200	27.50 ± 0.003	28.24 ± 0.022	52.63 ± 0.004

The IC_{50} of a sample is the concentration of the sample required to scavenge 50% of the peroxide in a system. It is used to evaluate the antioxidant capacity of a sample. The lower the IC_{50}, the better the antioxidant potential of the sample under examination [30]. Results showed that, hexane and methanol extracts demonstrated a significant antioxidant activity in concentration-dose dependent manner. The IC_{50} values of gallic acid (standard drug), hexane and methanol extracts ranged from 204.4 to 2872.0 µg/mL as shown in Table 4.

Table 4: IC_{50} of Hydrogen Peroxide Scavenging Activity

Sample	IC_{50} (µg/mL)
Standard (Gallic acid)	204.4
Hexane	2872.0
Methanol	1143.0

From the results, both methanol and hexane extracts which comprise of a mixture of compounds were not as potent as the gallic acid (standard drug), even though they are all good antioxidants.

3.3.3 Total antioxidant capacity (TAC)

Ascorbic acid also known as Vitamin C is an electron donor antioxidant and this property is responsible for all its known functions. Vitamin C is a potent reducing agent and scavenger of free radicals in biological systems. Vitamin C can both act as a strong, efficient and cheap antioxidant agent and, at the same time, behave as a radical promoter [31].

Concentrations of ascorbic acid ranging between 6.125 to 100 µg/mL showed antioxidant activity and mean absorbances between 0.059 ± 0.003 to 0.932 ± 0.002 at wavelength of 695 nm (Figure 2). The TAC values of the extracts were calculated by substituting the mean absorbances corresponding to the various extract concentrations into the linear equation (y = 0.0091x + 0.0818) of the mean absorbance versus concentration plot for the ascorbic acid (Figure 2), and finding their respective equivalence of ascorbic acid concentrations.

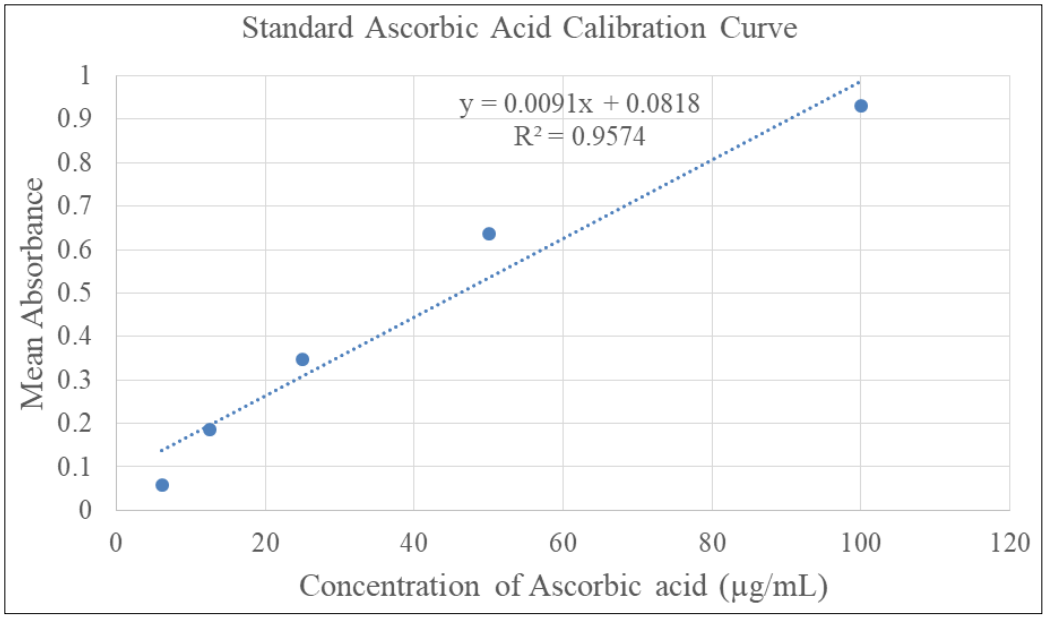

Fig 2: Absorbance of $PMo^{V}_{4}Mo^{VI}_{8}O_{40}^{7-}$ (Formed in ascorbic acid solution) against concentration of ascorbic acid solution.

The TAC was found to be proportional to the concentration of extract. TAC of the extracts were examined by Phosphomolybdenum method and the results were expressed as gram ascorbic acid equivalent per 100 grams (gAAE/100g). The gAAE/100g, represents the fraction of the plant extract that can act as ascorbic acid in 100 g of the extract. The hexane and methanol extracts had 17.389 and 21.563 gAAE/100g, respectively, (Table 5).

Table 5: Total Antioxidant Capacity of Hexane and Methanol extracts expressed as gAAE/100g

Extract	TAC (gAAE/100g)
Hexane	17.389
Methanol	21.563

TAC - Total Antioxidant Capacity;
AAE - Ascorbic Acid Equivalent

The reducing power of natural plant extracts might be strongly correlated with their antioxidant activities [32]. The antioxidant potency of both extracts could in part be attributed to the presence of polyphenols including flavonoids and tannins which have been shown to exhibit antioxidant activities [32]. Generally, the TAC increased with increasing concentration. The higher the TAC, the better the activity of the sample. All the extracts demonstrated appreciable antioxidant activities.

3.4 Antimicrobial Assay

3.4.1 Agar well diffusion

The antimicrobial activities of the extracts were determined at two concentration levels of 10.0 and 20.0 mg/mL for the agar well diffusion assay as shown in Table 6.

Table 6: Mean zones of inhibition (ZI) for hexane and methanol extracts of *P. peruviana* and standard drugs ciprofloxacin and clotrimazole in agar well diffusion assay

Sample/Drug	Conc. (mg/mL)	Zone of inhibition (mm) (mean ± SEM)				
		E. coli	*E. faecalis*	*P. aeruginosa*	*S. aureus*	*C. albicans*
Ciprofloxacin	0.05	26.7 ± 0.1	35.3 ± 0.3	33.3 ± 0.4	30.0 ± 0.5	NA
Clotrimazole	0.05	NA	NA	NA	NA	26.0 ± 0.5
Hexane	20.0	10.5 ± 0.1	11.0 ± 0.1	-	10.3 ± 0.1	-
	10.0	-	-	-	-	-
Methanol	20.0	15.5 ± 0.3	15.3 ± 0.1	17.3 ± 0.1	17.0 ± 0.3	14.3 ± 0.1
	10.0	11.5 ± 0.1	12.3 ± 0.1	13.3 ± 0.4	12.3 ± 0.4	12.3 ± 0.1

NA=Not Applicable, Diameter of cork borer = 10 mm

The agar well diffusion is carried out to test for the sensitivity of the organisms to the antimicrobial agent (plant extract). The diameter of the zone of inhibition determines the effectiveness of the extract against the microorganism. The larger the diameter, the greater the sensitivity of the microorganism to the extract. The sizes of the zone of inhibition are compared to standards to determine if the microorganism is sensitive or resistant to the plant extract.

From the results obtained, the lowest concentration at which the methanol and hexane extracts recorded a zone of inhibition was 10 and 20 mg/mL, respectively. No zone of inhibition was recorded for hexane at concentrations of 10.0 mg/mL. The methanol extract recorded the larger zones of inhibition and also at a concentration of 10.0 mg/mL was able to inhibit the growth of all the microbes to some extent. The hexane extract showed inhibition against *E. coli*, *E. faecalis* and *S. aureus* at a concentration of 20 mg/mL but no inhibition against *P. aeruginosa* and *C. albicans*. All the four tested bacteria were susceptible to the ciprofloxacin (standard drug) with the gram-positive bacteria *E. faecalis* showing the highest susceptibility. Methanol extract and clotrimazole (standard drug) showed activity against the fungus *C. albicans.*

3.4.2 Broth microdilution

The extracts showed broad spectrum antimicrobial activity against the tested organisms. The methanol extract showed a better antimicrobial activity (at MIC of 1.25 mg/mL to 5.00 mg/mL) against the test organisms than the hexane extract (at MIC of 2.50 to 25.00 mg/mL). The results are shown in Table 7.

Table 7: Minimum inhibitory concentrations (MIC) of extracts and reference drugs against test organisms

Test Organisms	Minimum Inhibitory Concentration (mg/mL)			
	Methanol	Hexane	Ciprofloxacin	Clotrimazole
E. coli	2.5	2.5	5.00×10^{-3}	NA
E. faecalis	1.25	2.5	0.625×10^{-3}	NA
P. aeruginosa	5.0	20.0	2.50×10^{-3}	NA
S. aureus	1.25	2.5	0.625×10^{-3}	NA
C. albicans	1.25	2.5	NA	1.25×10^{-3}

NA=Not Applicable

The results from the antimicrobial assay performed showed that the two extracts of *P. peruviana* stem-bark exhibited varying inhibitory effects against the five selected microorganisms (two Gram-positive, two Gram-negative and one fungus). The best results were observed with the use of the methanol extract against all the selected microorganisms. The minimum inhibitory concentrations (MICs) were between the range of 1.25 mg/mL to 5.00 mg/mL. The highest activity observed with the use of methanol extract was against *E. faecalis*, *S. aureus* and *C. albicans* with MIC of 1.25 mg/mL. The antimicrobial activity shown by the extracts could be attributed to the presence of terpenoids, carotenoids and tannins in the methanol and hexane extracts of *P. peruviana* which have been reported to exhibit antimicrobial activity [33].

3.5 Thin layer chromatography (TLC)

The number of components present in the extracts were determined by the analytical TLC method. The chromatographic spots which were representative of compounds in the various extracts were observed and their R_f values determined. Table 8 gives the results of the TLC analysis.

Table 8: TLC results of extract showing various components and their retardation factor using hexane/ethyl acetate (4:1) as mobile phase.

Components	Retardation factor, R_f	
	Hexane	Methanol
A	0.17	0.17
B	0.27	0.27
C	0.42	0.39
D	0.53	0.54
E	0.66	0.68
F	0.85	-
G	0.93	-

The hexane extract showed seven spots and methanol five spots with R_f values between 0.17 to 0.93 and 0.17 to 0.68, respectively. The number of spots indicating the separated components in the two extracts were less for methanol and more for hexane when compared to the phytoconstituents identified to be present in each stem-bark extract. This means that some of the components existed as isomers, did not elute due to the polarity of the mobile phase or co-eluted in mixtures and it may be necessary to employ two dimensional TLC, HPLC or column chromatography to achieve complete separation of the components.

4. Conclusions

The hexane and methanol extracts of *P. peruviana* showed the presence of varying secondary metabolites including saponins, tannins, terpenoids, steroids, flavonoids, glycosides and carotenoids. The study demonstrated that the hexane and methanol extracts of *P. peruviana* showed a variety of antibacterial, antifungal and antioxidant activities. This implies the extracts could be effective against infectious and oxidative-stress diseases, and could become a potential therapeutic agent for their treatment. Further studies are ongoing in our laboratory towards isolation, characterization, identification and determination of biological activities present in the stem-barks of *P. peruviana*.

Disclosure

Part of this work was presented as a poster at the "8th Ghana Science Association, Research Seminar and Poster Presentations" and "8th College of Health Sciences & 12th Convention of Biomedical Research Ghana joint Scientific Conference held at the Kwame Nkrumah University of Science and Technology, Kumasi, Ghana, in May and July 2019.

Conflicts of Interest

The authors declare no competing financial, professional, or personal interests that might have influenced the performance or presentation of the work described in this manuscript. The authors declare that there is no conflict of interests regarding the publication of this paper.

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