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Antimicrobial evaluation and phytochemical screening of aqueous and dichloromethane crude extracts of Kenyan *Physalis Peruviana* L (Cape Gooseberry)

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Abstract: *Physalis peruviana L a plant in Solanaceae family is used in folklore traditional medicine for treatment of bacterial, fungal and viral protozoal diseases. In this study aqueous and dichloromethane Physalis peruviana L extracts were evaluated for antimicrobial activity against some common bacterial and fungal isolates. The agar disc diffusion method was carried out to test antimicrobial activity against Staphylococcus aureus ATCC 25923, Bacillus cereus ATCC 11778, Escherichia coli ATCC 25922, Klebsiella pneumonia local isolate, Salmonella typhi ATCC 700931, Pseudomonas aeroginosa ATCC 27853, Candida albicans ATCC 90028 and Aspergillus flavus local isolate). All the plant extracts were also examined for Phytochemical components. Partial characterization of antimicrobial activity of the most active extract (dichloromethane leaf extracts) at 250 mg/ml was carried out at different pH and temperature ranges. The results on phytochemical screening demonstrated the presence of tannins, saponins, steroids, flavonoids, while anthraquinone was lacking in all extracts. Most of the plant extracts (87.5%) exhibited various inhibitory effects to test microbes while only a few (12.5%) did not show any antimicrobial activity. Statistical analysis for intra-group inhibitory activity amongst various extract concentrations demonstrated significant differences ($P<0.01$). The MIC and MMC of the various plant extracts ranged from 3.9 mg/ml to 62.5 mg/ml. The increase in pH and temperature led to a decrease and an increase in antimicrobial activity respectively. These results indicate that all plant parts of P. peruviana could be potential sources of antimicrobial agents with dichloromethane leave extracts being the most potent.*

Keywords*: Antimicrobial, Phytochemical, Aqueous extracts, Dichloromethane extracts, Physalis peruviana L.*

I. Introduction

The use of medicinal or herbal plants in treatment of infectious diseases has been known since the ancient times. The activities of the plant extracts is probably due the presence of various bioactive phytochemicals [1]–[3]. There has been a rise in cases of infectious diseases due to development of antimicrobial resistance by various micro-organisms. This has necessited prompt investigations to discover new and more efficient antimicrobials for treating infections and to prevent further spread of the resistant micro-organisms [3]. According to aduol *et al* [3], infectious diseases have a large impact in the world, resulting to mortalities of one quarter of the world population. In Kenya as in other developing countries, there has been a tendency to incorporate herbal medicine in health systems. This has resulted to low costs in accessing health services especially to the poor large rural populations [4], [5].

The World Health Organization stipulates that medicinal plants could be the best source of various drugs. In developed countries, approximately 80% of the people use drugs obtained from medicinal plants, while 80% of developing countries use herbal medicine to address their disease concerns [6]. It is important to carry out more research in medicinal plants in order to validate their efficacy and also their safety profiles so as to come up with more noble drugs to overcome the problem of disease resistant microbes [6], [7].

Physalis peruviana L (Cape gooseberry) is a plant in the family of solanaceae and its fruits have been reported to have high nutritional value with high contents of vitamins, antioxidants and minerals [8]. Some research undertakings have revealed its medicinal properties including antibacterial, anti-inflammatory, antiasthma, anticancer, induction of apoptosis, boosting of immune system, elimination of parasites and treatment of skin ailments [2], [8]–[10]. In a study carried out in Kagera region, north western Tanzania fruit juice of *Physalis peruviana* L was found to be used for treatment of malaria [11].

The aqueous leaf extracts of *Physalis peruviana* L were also found to have antibacterial activity against three gram negative bacteria (*Eschericia coli, Proteous vulgaris Seratia mersescens*) and three gram positive bacteria (*Bacillus subtilis, Micrococcus kristinae* and *Staphylococcus aureus)* [12]. *Physalis angulata* L a plant in the same family has been associated with antibacterial, anticancer, and immunostimulant properties [13].

The purpose of this study was to evaluate the antimicrobial activity of crude aqueous and dichloromethane extracts of *Physalis peruviana* L against six bacterial isolates (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* local isolate, *Salmonella typhi* ATCC 700931, *Pseudomonas aeroginosa* ATCC 27853) and two fungal isolates (*Candida albicans* ATCC 90028 and *Aspergillus flavus* local isolate). The extracts were also screened for the phytochemical components.

II. Materials and Methods

2.1 Collection of plant materials and authentication

The plant materials were collected from Nyeri County [0°25`0``South, 36°57`0``East] located in Central part of Kenya. Nyeri is 162 Km North of Nairobi. This area is known to have good reserves of *P. peruviana* L. The plan whole plant materials were collected in 2013 and identified by National Museums of Kenya Botanists and a Voucher specimen number EAH001PK deposited at the Department of Medical Laboratory Sciences of Jomo Kenyatta University of Agriculture and Technology, Kenya. Various parts; fruits, leafs, stem and roots were separated, dried under shade and pulverized in a hammer mill fitted with a sieve of 0.5mm pore.

2.2 Sources of chemicals and media

Dichloromethane was purchased from Fisher Scientific, UK, ltd. Mueller Hinton agar, Nutrient broth, Potato dextrose broth and Sabourauds dextrose agar (SDA) were purchased from UK, Biotech Laboratories ltd, while DimethylSulfoxide (DMSO) was procured from sigma (Pool, Dorset, England).

2.3 Preparation of extracts

Preparation of plant extracts was carried out using methods described by Ubulom [14]. Pulverished plant parts were soaked separately in distilled water and dichloromethane, for 72h with stirring at regular intervals. The extracts were repeatedly filtered using sterile whatman No. 1 filter paper [12]. The aqueous filtrates were freeze dried, while the dichloromethane extracts were concentrated under vacuum in a rotary evaporator.

The percentage yield was determined using the method previously applied by Ogila [15] as follows; Percentage yield = Weight of extract/Weight of ground material X 100.

For identification purposes the extracts were assigned codes and interpreted as follows; APPL = aqueous extracts of *P. peruviana* leaf; APPS = aqueous extracts of *P. peruviana* stem; APPF = aqueous extracts of *P. peruviana* fruit, APPR = aqueous extracts of *P. peruviana* root; DPPL = dichloromethane extracts of *P. peruviana* leaf; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPF = dichloromethane extracts of *P. peruviana* fruit; DPPR = dichloromethane extracts of *P. peruviana* root. All the extracts were kept desiccated at 4^oC until use.

2.4 Antibacterial and antifungal evaluation

In this study there were six bacterial isolates and two fungal isolates. Four of the bacterial isolates were gram negative (*Escherichia coli* ATCC 25922, *Klebsiella pneumonia* local isolate, *Salmonella typhi* ATCC 700931, *Pseudomonas aeroginosa* ATCC 27853), while the other two were gram positive (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778). There were two fungal isolates namely; *Candida albicans* ATCC 90028 and *Aspergillus flavus* local isolate. The organisms were obtained from culture maintained in the department of Medical Laboratory Science, Jomo Kenyatta University of Agriculture and Technology. Antibacterial and antifungal inhibitory activities were tested using a modified disc diffusion method as applied by Ogila *et al* [15]. To obtain active cultures for antibacterial and antifungal activity, a loopful of test organisms from stock cultures were transferred to test tubes of Muller Hinton and Sabouraud dextrose broth for bacteria and fungi respectively. The tubes were incubated overnight at 37^oC. Dilutions for the cultures were made in each respective broth by comparing their turbidity with McFarland standard so as to achieve values corresponding to $2x10^6$/ml and $2x10^5$/ml colony forming units/spore forming units for bacteria and fungi respectively. The extracts were dissolved in DMSO in order to obtain concentrations ranging from 31.25 mg/ml to 250 mg/ml. Sterile filter paper discs (whatman No. 1) of 6mm diameter were impregnated with the different concentrations of crude extracts. Discs impregnated with distilled water and dichloromethane served as negative controls. Ciprofloxacin 10 µg/ml and Amphotericin-B 10 µmg/ml were used as standards for antibacterial and antifungal drugs.

Antibacterial and antifungal activity for each extract was carried out by spreading 100 µl of inoculums of each test organism on the recommended specific medium, Muller Hinton and Sabourauds for bacterial and fungal isolates respectively. The filter discs containing each extract was aseptically placed on inoculated plates. The plates were allowed to stand for about 10 min for diffusion of extract to take place. The plates for antibacterial activity were incubated at 37^0C for 24 h, while plates for antifungal activity were incubated at room temperature (25^0C) for 48 h. The antibacterial and antifungal activity was determined from the formation of inhibition zones surrounding the disc containing the extract in millimeters using a vernier calipers. The experiment was carried in triplicates and results expressed as mean inhibition zones (mm) ± standard error of three triplicate readings.

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC)

To analyze for MIC and MMC the method previously described by Doughari [16] was used. The MIC was carried out by transferring 0.5ml different concentrations (3.9, 7.8, 15.6, 31.2, 62.4, 124.8) of the extract to sterile bottles containing Nutrient Broth (NB) or Potato Dextrose Broth (PDB) for bacterial and fungal isolates respectively. Each of the test bacteria suspension matched at 0.5 MacFarland turbidity standard or the fungal isolates at 10^6 spores/ml was introduced into a corresponding tube containing 0.5ml reconstituted NB or PDB for bacteria or fungi respectively. A set of bottles containing bacteria or fungi for each specific broth were used as controls. All the cultures were incubated at 37^oC for 24 h (bacteria), or at room temperature (25^0C), 48 h in the dark for fungal isolates. After incubation at the specified conditions, the bottles containing concentrations with no visible growth were considered as MIC.

The MMC test was carried out by inoculating Muller Hinton Agar or Sabarauds dextrose Agar plates with a loopful of culture from tubes that had no visible growth. Plates were incubated again at the right temperature and time conditions and examined for growth. Any concentration that exhibited no growth was taken as the MMC.

2.6 Partial characterization of dichloromethane *P. peruviana* L extracts

The most bioactive extract (250mg/ml) *P. peruviana* L leaf extracts) from the antimicrobial disc diffusion inhibitory tests was partially characterized for antimicrobial activity at different temperature and pH ranges using method previously employed by Doughari [16]. Five milliliters of 250mg/ml of dichloromethane leaf extract was prepared into test tubes and treated at 25, 50, 70 and 100^oC in a water bath for 1/2h and tested for antimicrobial activity with the tube at 25^oC (room temperature) serving as a control.

Determination of *P. peruviana* L dichloromethane leaf extract effects on pH was carried out by exposing the extracts at pH ranges; 3, 5, 7, 9 and 12 using 1N HCL and 1N NaOH solutions for acidic and alkaline adjustments respectively. The extracts were adjusted to the respective pH values using 1N HCL and 1 N NaOH and tested for antimicrobial activity with pH 5 of the untreated sample serving as the control.

2.7 Phytochemical screening

The extracts were analyzed for the presence of tannins, alkaloids, anthraquinones, flavonoids, steroids and saponins using methods applied by Ubulom [14].

2.8 Statistical analysis

Microsoft Excell^R was used as the tool to enter and capture data. The data was subjected to SPSS 15.0 package for statistical analysis. The antimicrobial disc diffusion tests results were analyzed for intra-group extract concentration comparisons using Analysis Of Variance (ANOVA). P-value < 0.05 was considered as significant, while a P-value of < 0.01 as highly significant.

III. Results

3.1 Percentage Yields Of Various P. Peruviana L Plant Extracts

The percentage yield for aqueous and dichloromethane plant extracts ranged from 0.5 to 10% (Table 1). These results indicate that the more polar aqueous solvent was more effective in extracting plant materials as compared to the less polar dichloromethane solvent. The preliminary phytochemical analysis of aqueous and dichloromethane extracts are as shown (Table 2). The aqueous and dichloromethane leaf extracts revealed the presence of tannins. Saponins were detected in all other extracts except in aqueous leaf and fruit extracts. Dichloromethane leaf and fruit extracts revealed the presence of steroids. The study revealed presence of flavonoids in most of the extracts except in aqueous fruit and dichloromethane stem extracts. Alkaloids were not detected in aqueous and dichloromethane extracts, while anthraquinones were absent in all the *P. peruviana* L extracts.

The highest concentration of phytochemical constituents were recorded as follows; tannins in both aqueous and dichloromethane leafs, flavonoids in dichloromethane fruit, alkaloids in both aqueous stem and dichloromethane extracts.

Table 1: Percentage yield of aqueous and dichloromethane crude extracts of *Physalis peruviana*

Plant extract	Amount of ground part in (g)	Yield of extract (g)	% Yield of the extract (g)
APPL	100	10	10
APPS	100	3	3
APPF	120	0.6	0.5
APPR	100	6	6
DPPL	40	1	2.5
DPPS	40	1.34	3.4
DPPF	120	0.83	0.7
DPPR	120	4.3	3.6

Key: g: = gram, % = percent, APPL = aqueous extracts of *P. peruviana* leaf, APPS = aqueous extracts of *P. peruviana* stem, APPF = aqueous extracts of *P. peruviana* fruit, APPR = aqueous extracts of *P. peruviana* root, DPPL = dichloromethane extracts of *P. peruviana* leaf, DPPS = dichloromethane extracts of *P. peruviana* stem; DPPF = dichloromethane extracts of *P. peruviana* fruit; DPPR = dichloromethane extracts of *P. peruviana* root.

Table2: Phytochemical components of aqueous and dichloromethane of *Physalis peruviana* extracts.

Plant extract	**Phytochemical components**					
	Tannins	**Saponins**	**Steroids**	**Flavonoids**	**Alkaloids**	**Anthraquinone**
APPL	+++	-	-	+	+	-
APPS	+	++	-	++	+++	-
APPF	-	-	-	-	-	-
APPR	+	++	-	+	++	-
DPPL	+++	++	++	++	+++	-
DPPS	_	++	-	-	+	-
DPPF	-	++	++	+++	-	-
DPPR	-	++	-	+	+	-

Key: APPL = aqueous extracts of *P. peruviana* leaf, APPS = aqueous extracts of *P. peruviana* stem, APPF = aqueous extracts of *P. peruviana* fruit, APPR = aqueous extracts of *P. peruviana* root, DPPL = dichloromethane extracts of *P. peruviana* leaf, DPPS = dichloromethane extracts of *P. peruviana* stem, DPPF = dichloromethane extracts of *P. peruviana* fruit, DPPR = dichloromethane extracts of *P. peruviana* root.
+++ (present in high concentration), ++ (moderately present), + (trace), - (absent)

3.2 Antimicrobial Activity

Most of the plant extracts (87.5%) exhibited various inhibitory effects to the test microbes, while only a small percentage of plant extracts (12.5%) did not show any antimicrobial activity to the tested microbes. The extracts that had no antimicrobial activities were; the aqueous stem to both *Klebsiella pneumonia* local isolate, *Salmonella. typhi* ATCC 700931 , *Pseudomonas aeroginosa* ATCC 27853 and *E.coli*. The aqueous leaf extracts didn't exhibit inhibitory effect to both *Klebsiella. pneumonia local isolate* and *Salmonella. typhi* ATCC 700931. *P. peruviana* L fruit extract didn't demonstrate any inhibitory effects to *E.coli, Klebsiella pneumonia* local isolate and *Salmonella. typhi* ATCC 700931, while the dichloromethane fruit had no activity to *Pseudomonas aeroginosa* ATCC 27853.The highest antimicrobial activity was recorded from the dichloromethane leaf extracts in *Bacillus cereus* ATCC 11778 (14.03± 0.03 mm), while the lowest antimicrobial activity was recorded from dichloromethane leaf extracts to *Eschericia coli* ATCC 25922 (1.13±0.07). Statistical analysis for intra-group inhibitory activity amongst various extract concentrations demonstrated high significant differences ($P<0.01$). The antimicrobial activity increased with increase in extract concentration.

The results also indicated that the dichloromethane leaf extracts generally exhibited higher antimicrobial activity to tested microbes as compared to other *P. peruviana* L extracts.

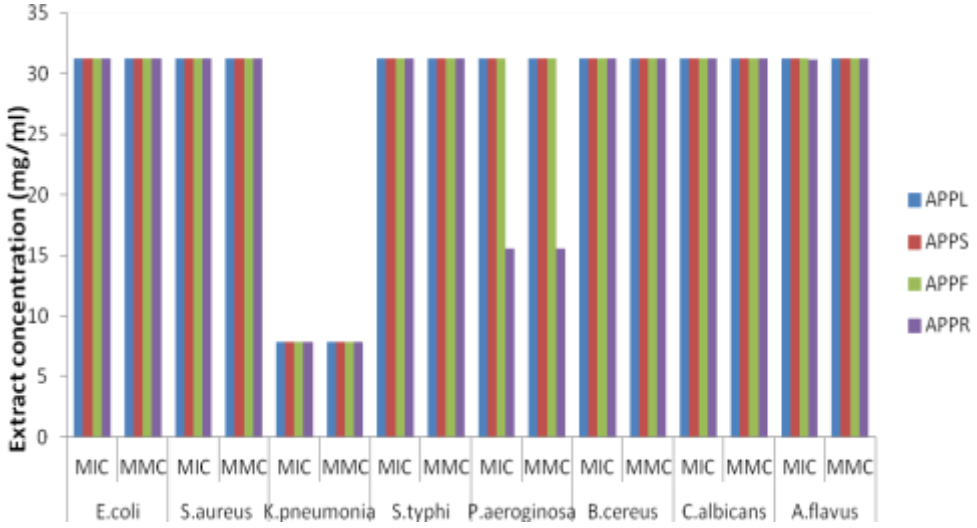
Table 3: Antimicrobial activity of aqueous and dichloromethane extracts. The values are expressed as mean inhibition zones (mm) ± standard error of the mean of three triplicate readings with Ciprofloxacin and Amphotericin B as reference standards.

Plant part	**Conc. mg/ml**	*E. coli*	S. aureus	*K.pneumoniae*	*S. typhi*	*P.aeroginosa*	*B. cereus*	*C.albicans*	*A. flavus*	p-value
APPL	31.25	-	-	-	-	-	-	-	-	0.000
	62.5	-	3.13±0.03	-	-	2.20±0.06	1.07±0.07	-	-	
	125	-	4.17±0.03	-	-	3.10±0.06	2.05±0.05	3.13±0.03	2.10±0.06	
	250	1.13±0.07	4.50±0.06	-	-	4.03±0.03	2.50±0.06	3.80±0.12	4.17±0.12	
APPS	31.25	-	1.37±0.09	-	-	-	-	-	-	0.000
	62.5	-	2.00±0.00	-	-	-	2.0±0.00	-	-	
	125	-	3.13±0.07	-	1.20±0.06	-	2.0±0.00	2.00±0.00	-	
	250	-	4.20±0.06	3.13±0.03	2.10±0.06	-	2.37±0.15	4.07±0.07	2.17±0.12	
APPF	31.25	-	1.13±0.03	-	-	-	-	-	-	0.000
	62.5	-	2.17±0.09	-	-	2.03±0.03	-	-	-	
	125	-	3.20±0.12	-	-	3.13±0.13	1.20±0.00	2.07±0.03	2.10±0.07	
	250	-	4.10±0.06	-	-	3.73±0.13	2.20±0.12	3.40±0.00	4.30±0.10	
APPR	31.25	-	3.30±0.15	1.13±0.13	-	-	4.03±0.03	-	-	0.000
	62.5	-	5.20±0.06	2.13±0.13	-	-	6.03±0.03	3.27±0.07	3.07±0.07	
	125	1.04±0.04	6.6±0.12	2.57±0.03	2.33±0.03	2.20±0.10	7.00±0.00	3.70±0.07	4.23±0.09	
	250	2.40±0.31	7.20±0.09	4.17±0.09	3.43±0.03	3.50±0.06	8.20±0.12	3.97±0.09	4.70±0.07	
DPPL	31.25	4.1±0.09	5.60±0.12	4.03±0.03	3.17±0.09	5.07±0.07	5.17±0.03	6.43±0.28	7.07±0.07	0.000
	62.5	6.03±0.03	6.03±0.03	5.10±0.06	4.20±0.12	5.27±0.13	7.20±0.12	8.07±0.07	9.07±0.07	
	125	5.50±0.05	7.2±0.06	6.20±0.12	4.80±0.20	6.03±0.03	8.70±0.35	9.67±0.33	11.33±0.33	
	250	6.0±0.00	11.00±0.05	8.03±0.03	5.20±0.12	7.10±0.06	14.03±0.03	11.33±0.33	13.40±0.31	
DPPS	31.25	2.37±0.32	3.90±0.15	3.07±0,07	2.37±0.09	4.07±0.07	4.07±0.07	5.00±0.00	6.03±0.33	*0.000
	62.5	2.57±0.22	5.06±0.07	4.03±0.03	3.23±0.09	5.23±0.03	6.07±0.07	6.33±0.33	8.67±0.33	
	125	3.10±0.58	5.93±0.07	4.80±0.3	3.67±0.07	5.80±0.20	8.33±0.33	9.00±0.00	10.07±0.00	

	250	4.23±0.19	6.37±0.09	5.37±0.19	4.03±0.03	7.13±0.47	12.33±0.33	9.33±0.33	12.10±0.10	
DPPF	31.25	-	2.17±0.09	-	-	-	2.07±0.07	-	-	0.000
	62.5	-	3.07±0.07	-	-	-	3.10±0.10	-	3.03±0.03	
	125	-	4.00±0.00	3.10±0.10	-	-	4.07±0.07	2.20±0.06	4.07±0.07	
	250	250	2.43±0.28	4.60±0.00	3.37±0.03	2.27±0.03	-	5.67±0.33	4.07±0.07	
DPPR	31.25	-	2.57±0.22	-	-	-	3.07±0.06	-	4.10±0.06	0.000
	62.5	1.3±0.06	3±0.00	-	-	4.27±0.13	3.50±0.06	4.07±0.07	6.17±0.12	
	125	2.37±0.03	3.50±0.06	3.03±0.03	1.2±0.06	4.67±0.33	4.03±0.03	5.17±0.12	7.27±0.13	
	250	3.13±0.03	4.07±0.07	4.00±0.00	2.27±0.03	5.30±0.06	8.03±0.03	6.03±0.33	8.10±0.06	
Cipro	10µg/ml	32.13±0.06	27.10±0.0	24.66±0.33	28.93±0.9	28.46±0.29	26.66±0.3	NA	NA	NA
Amphot- B	10µg/ml	NA	NA	NA	NA	NA	NA	29.10±0.5	29.33±0.33	NA

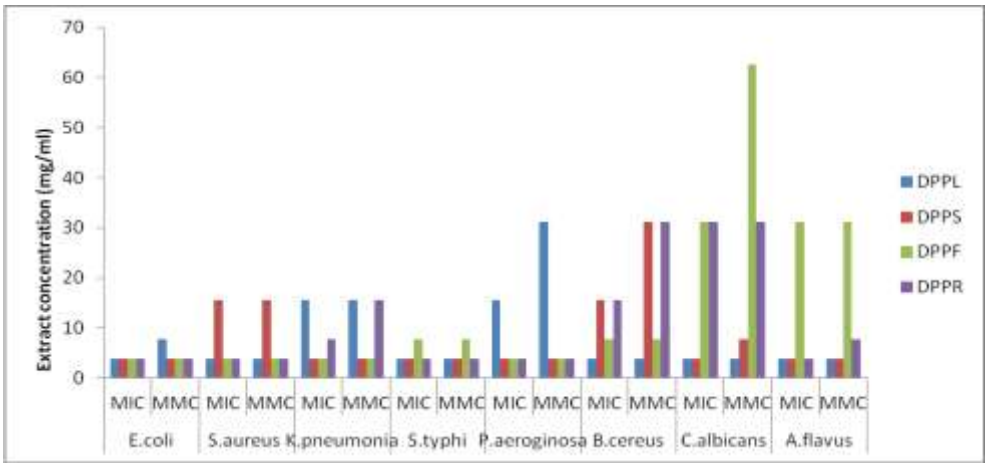
Key: APPL = aqueous extracts of *P. peruviana* leaf; APPS = aqueous extracts of *P. peruviana* stem; APPF = aqueous extracts of *P. peruviana* fruit; APPR = aqueous extracts of *P. peruviana* roots; DPPL = dichloromethane extracts of *P. peruviana* leaf; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPF = dichloromethane extracts of *P. peruviana* fruit; DPPR = dichloromethane extracts of *P. peruviana* root; *0.000 signifies that the p-value for intra-group comparisons in DPPS extract were at 0.000 except for *E.coli* which was at 0.001; - no antimicrobial activity; Cipro = Ciprofloxacin and Amphot-B = Ampotericin B, NA = not applicable to the microbe or statistical analysis.

The minimum inhibitory concentration (MIC) of the various extracts ranged from 3.9 to 31.2 mg/ml, while the minimum microbicidal concentration (MMC) ranged from 3.9 to 62.4 mg/ml.



Key: APPL = aqueous extracts of *P. peruviana* leaf; APPS = aqueous extracts of *P. peruviana* stem; APPF = aqueous extracts of *P. peruviana* fruit; APPR = aqueous extracts of *P. peruviana* root.

Figure 1: Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of aqueous *P. peruviana* L extracts against tested microbes.



Key: DPPL = dichloromethane extracts of *P. peruviana* leaf; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPF = dichloromethane extracts of *P. peruviana* fruit; DPPR = dichloromethane extracts of *P. peruviana* root.

Figure 2: Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of dichloromethane *P. peruviana L* extracts against tested microbes.

Partial characterization of *P. peruviana* L extracts revealed a rise and a fall in antimicrobial activity as a result of increase in temperature or pH respectively.

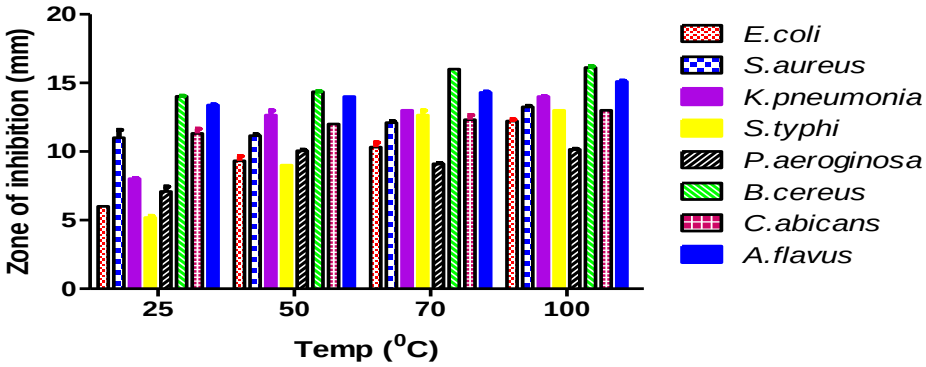


Figure3: Effect of temperature on the antimicrobial growth of *P. peruviana* dichloromethane leaf extracts (250 mg/ml). Results are expressed as inhibition zones of three triplicate readings.

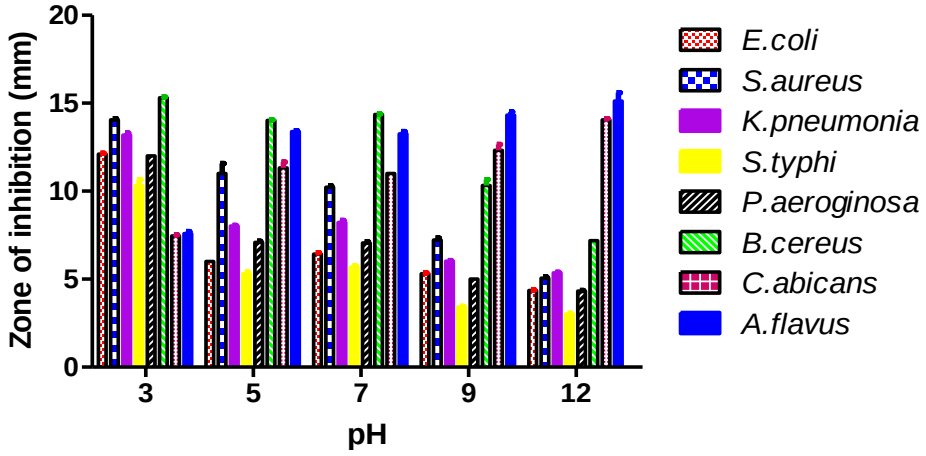


Figure 4: Effect of pH on the antimicrobial growth of *P. peruviana* dichloromethane leaf extracts (250 mg/ml). Results are expressed as inhibition zones of three triplicate readings.

V. Discussion

Physalis peruviana L a herbaceous plant in the Solanaceae family has been in use in folklore medicine for treatment of various microbial infections [2], [8], [12], [17]. The 5 main factors that determine the antimicrobial activity of plant extracts are the quality of constituents of plant components, the amount used, pH value, temperature and the type of microbe used [1].

The yield of aqueous and dichloromethane of *Physalis peruviana* L extracts ranged from 0.5 – 10% and 0.7 – 3.6% respectively. These results were comparable with those of other researchers where the more polar solvents yielded higher extracts [7], [19]. The use of crude extracts for antimicrobial activity investigations have been reported to possess potential success compared to screening of pure compounds from natural products [15]. *Physalis peruviana* L extracts have been reported to possess antimicrobial activity [2], [10]–[12].

In the present study the agar disc diffusion method was used to evaluate the aqueous and dichloromethane extracts of *P. peruviana* L leaf, stem, fruit and root against six bacterial isolates (*Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778, *Escherichia coli* ATCC 25922, *Klebsiella pneumonia* local isolate, *Salmonella typhi* ATCC 700931, *Pseudomonas aeroginosa* ATCC 27853 and 2 fungal isolates (*Candida albicans* ATCC 90028 and *Aspergillus flavus* local isolate). Ciprofloxacin and amhotericin B were used as standard drugs for antibacterial and antifungal respectively. Disc diffusion results revealed that, the intra group extract concentrations exhibited high significant antimicrobial activity ($P<0.01$). These results are in agreement with a report on antimicrobial properties of some wild leafy vegetables that revealed aqueous *P. peruviana L leaf extracts* being active against; *Bacillus subtilis*, *Micrococcus kristinae*, *Staphylococcus aureus* ATCC 25923, *Proteus vulgaris* and *Serratia marcescens* [12]. *Staphylococcus aureus* had a general higher antimicrobial activity while *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia* exhibited the lowest antimicrobial activities. There was no antimicrobial activity in aqueous stem and fruit extracts against *Escherichia coli* ATCC 25922, aqueous fruit *extracts against Klebsiella pneumonia* local isolate and *Salmonella typhi* ATCC 700931and no activity was demonstrated in aqueous stem extracts against *Pseudomonas aeroginosa* ATCC 27853. Most of the *P. peruviana* L dichloromethane leaf extracts in this study revealed antimicrobial activity with the highest activity (14.03±0.03 mm) being on leaf extracts against *Bacillus cereus* ATCC 11778 and the lowest activity was exhibited in *Salmonella typhi* ATCC 700931 (2.27±0.03 mm) from both dichloromethane fruit and root extracts. However, there was no activity in dichloromethane fruit extracts against *Pseudomonas aeroginosa* local isolate.

In a study carried out on essential oils [13], from *Physalis angulata* L a related plant species to *P. peruviana* L; the aerial parts were reported to posses antimicrobial activity against *B. subtilis* and *K. pneumonia*; the roots were active active against *K. pneumonia*, while aerial parts were antifungal to *C.albicans, C. stellatoides* and *C. turulopsis*. These findings are closely similar to the present study in that, *P.peruviana* L *dichloromethane* leaf and root extracts were inhibitory to *Pseudomonas aeroginosa* ATCC 27853, *Klebsiella pneumonia* local isolate, and *Candida albicans* ATCC 90028. The data on inhibition zones obtained from this study is in support with claims that, inhibition zone diameters are dependent on the bacteria strain and species used [7].

The minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) was determined by microdilution assays on the various *P. peruviana* L extracts. MIC and MMC values are used to determine the treatment outcomes of antimicrobial agents. The results of the current study are in agreement in that higher values of MIC and MBC were associated with low activity of the antimicrobial agent, while low MIC and MBC values denotes higher activity to the microbial agent [6], [7]. In all the aqueous extracts of *P.peruviana* L, MICs and MMCs of 31.2 mg/ml were recorded against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Candida. albicans* ATCC 90028 and *Aspergillus flavus* local isolate. The aqueous *P. peruviana* L leaf, stem and fruit extracts exhibited same MIC and MBC values of 32.2 mg/ml to *Pseudomonas aeroginosa* local isolate, while a MIC an MBC of 15.6 mg/ml of aqueous root extracts was recorded against *Pseudomonas aeroginosa* local isolate.

These results differ from those of a previous study [12], in that MICs for aqueous *P. peruviana* leaf extracts against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were recorded as 1.0 and 5.0 mg/ml respectively, while in the current study the MICs for the 2 organisms were both at 31.2 mg/ml. These differences can be attributed to the bacterial strain used or the type of extraction method employed.

The dichloromethane *P.peruviana* L extracts exhibited varying MIC and MMC values with leaf extracts exhibiting the lowest MIC and MMC values ranging from 3.9 mg/ml to15.6 mg/ml and 3.9 mg/ml to 31.2 mg/ml respectively. These results indicate that dichloromethane had better extraction properties compared to the more polar aqueous solvent and also that dichloromethane leaf extracts posses better antimicrobial activity compared to the other *P.peruviana* L extracts .Partial characterization of dichloromethane leaf extracts (250 mg/ml) revealed a general increase in antimicrobial activity with an upward adjustment of temperature and vice versa for the upward adjustment in pH. This characterization is in agreement with another previous research finding where an increase of plant extract temperature and a decrease in pH raised and lowered antimicrobial activity respectively [6].

Although a majority of people use the *p. peruviana* L fruits as supplementary diet and associate it with medicinal value, both the aqueous and dichloromethane fruit extracts were found to be weaker against tested microbes compared to the other extracts.

Phytochemical components in plant extracts have been found to posses curative effects to microbial infections [20]. This study revealed the presence of tannins in leaf, stem and root aqueous extracts, while flavonoids and alkaloids were present in leaf, stem and root aqueous extracts. Dichloromethane extracts revealed the presence of tannins in leaf, stem, fruit and root extracts. Steroids and flavonoids were detected in both leaf and fruit extracts, while alkaloids were detected in leaf, stem and root extracts. They were not detected in both the aqueous and dichloromethane extracts. The presence of these phytochemical components in *P. peruviana* L extracts can be the reason of its use in traditional folklore medicine. Flavonoids have been found to be effective against a wide range of microbes [19]. Their mechanism of action is thought to be from their ability to form complexes with exracellular and soluble protein and bacterial cell walls. Tannins are associated with formation of irreversible complexes with nucleophilic aminoacids in proteins many times leading to inactivation of proteins, loss of function and death of microorganisms.

Alkaloids have been attributed to posses microbicidal properties to protozoas such as *Giardia* and *Entamoeba* species. The mechanisms of action of the highly aromatic alkaloid such as berberine and harmane is believed to be in their ability to interact with DNA. The saponins have been reported to posses anti-inflammatory, antiviral and plant defense activities [20]. Steroids and in particular corticosteroid has been in use for treatment of immune mediated diseases such as psoriasis due to their immunosuppressive, anti-proliferative and anti-inflammatory properties [21]. The presence of the various phytochemical compounds present in *P. peruviana* L could be associated with the antimicrobial activities associated with the extracts.

VI. Conclusion

The broad antimicrobial activity to both gram positive, gram negative bacterial and fungal isolates demonstrated in most of the extracts of *P.peruviana* L may be utilized to develop broad spectrum antimicrobials. The leafs of *P. peruviana* L were found to be the most potent on antimicrobial activity and therefore cultivation of this plant should be encouraged so as to facilitate more research for discovery of more noble antimicrobial agents. There is need to carry out more studies for isolation of pure compounds from this plant.

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