academicJournals

Vol. 9(11), pp. 370-378, 17 March, 2015
DOI: 10.5897/JMPR2015.5758
Article Number: A2692C951781
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

In vitro antimicrobial activity of "Antibact", an herbal medicinal product against standard and clinical bacterial isolates

Felix Charles Mills-Robertson[1], Cynthia Igbukolu Onyeka[2], Samuel Crowther Kofi Tay[2] and Williams Walana[3]*

[1]Department of Biochemistry and Biotechnology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Department of Clinical Microbiology, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[3]Department of Clinical Laboratory Sciences, School of Medicine and Health Sciences (SMHS), University for Development Studies (UDS), Tamale, Ghana.

Received 5 February, 2015; Accepted 9 March, 2015

***In vitro* antimicrobial activities of ethanol and aqueous "Antibact", herbal products consisting of a combination of the leaves and branches of four different plants were evaluated against twenty one pathogenic bacteria. Saponins, reducing sugars, phenolics, polyuronides, and triterpenes were the major phyto-constituents of both the aqueous and ethanol "Antibact". The LD_{50} analysis revealed the products were safe (LD_{50}>5000 mg/kg bodyweight) for *in vivo* use. All the isolates (100%) were resistant to at least five of the 12 antibiotics used in the study. In total, the aqueous "Antibact" inhibited the growth of 5 out of the 21 (23.81%) microbes used with an average zone of inhibition of 9.73 ± 0.35 mm. Thirteen (61.90%) out of the 21 microbes used were susceptible to the ethanol "Antibact", registering an average inhibition zone of 10.80 ± 0.18 mm. In the case of the minimum inhibitory concentration (MIC), the aqueous "Antibact" exhibited MIC range of 4.00 to 32.00 mg/ml and 0.50 to 8.00 mg/ml, while the ethanol "Antibact" recorded MIC range of 2.00 to 8.00 and 1.00 to 2.00 mg/ml for the wild and standard strains, respectively. The average minimum bactericidal concentration (MBC) for the aqueous "Antibact" was 32.00 mg/ml while the ethanol "Antibact" had MBC range of 4.00 to 16.00 mg/ml and 4.00 to 8.00 mg/ml for the wild and standard strains, respectively. In conclusion, both "Antibact" were safe for human use and effective against some pathogenic bacteria *in vitro*. However, ethanol "Antibact" showed better antimicrobial activity.**

Key words: Antibact, antimicrobial activity, clinical isolate, drug-resistant.

INTRODUCTION

Medicinal plant may be defined as any plant whose some or all of its parts contain active compounds which can be used in the treatment or management of a disease. In the last decade, there have been global upsurge in the use of

*Corresponding author. E-mail: walanawilliams@yahoo.com.

traditional medicine (TM), and complementary and alternative medicines (CAM) in both developed and developing countries. Currently, TM and CAM play increasingly important role in health care and health sector reform globally. Hence, the safety and efficacy, as well as the quality control of TM and CAM have become important concerns for both health authorities and the public (WHO, 2005).

In Africa, up to 80% of the populations in rural areas depend on traditional medicine to meet their primary health care needs, while in India the corresponding figure is 65% (WHO, 2002). These figures are expected to go up in recent years. Contrary to the presumption that the 21st century Ghanaian care less and knows less about herbal medicine and its role in the general wellbeing of Ghanaians, studies conducted by corporate bodies and individuals proved otherwise (Addo, 2007; Darko, 2009). It is also a known fact that, TM is the first choice of healthcare treatment for more than 80% of Africans suffering from high fever and other common ailments (Matur et al., 2009).

Resistance to antibiotics is a serious worldwide problem which is increasing and has implications for morbidity, mortality, and health-care both in hospitals and in the community (Franco et al., 2009). For decades, antimicrobial drugs have proven useful for the treatment of infectious diseases but lately most bacteria are inherently resistant to newly developed antimicrobial agents (Newman et al., 2006). The emergence of the acquired resistance to antimicrobial drugs has been observed in almost all pathogenic bacteria (Newman et al., 2006) and the emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious diseases (Gibbons, 2005; Mathias et al., 2000). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance to many, and in some cases all effective first-line antibiotics (Mills-Robertson et al., 2009). Hence, there is need to look for alternative strategies for the management of resistant bacteria and one of the possible strategies towards this objective involves rational localization of bioactive phytochemicals which have antibacterial activity and this may be one of the important approaches for the containment of antibiotic resistance (Gottlieb et al., 2002).

This study therefore investigated the antimicrobial efficacy and safety of “Antibact”, herbal medicine products comprising of four plants namely: *Phyllanthus fraternus* G.L. Webster (Family Euphorbiaceae), *Hoslundia opposita* Vahl. (Family Lamiaceae), *Psidium guajava* L. (Family Myrtaceae) and *Cymbopongon citrates* (CD) Stapf (commonly called lemon grass). Studies have shown that these plants have varied degrees of antimicrobial activities and antioxidant properties (Mehta et al., 2014; Koffuor and Amoateng, 2011), but the combined effects of the plants have not been studies hence the need for the present study.

MATERIALS AND METHODS

Study site and plant

The study was carried out at the Microbiology Department of the Centre for Plant Medicine Research (CPMR) in Mampong-Akuapem. It is the main institute in Ghana where herbal products are certified for use before it is registered by the Food and Drugs Board of Ghana. All the medicinal plants including *Phyllanthus fraternus* G.L. Webster (Family Euphorbiaceae), *Hoslundia opposita* Vahl. (Family Lamiaceae), *Psidium guajava* L. (Family Myrtaceae) and *Cymbopongon citrates* (DC.) Stapf (commonly called lemon grass) used for the formulation of “Antibact” were identified and collected by a taxonomist at the Plant Development Department (PDD) of the CPMR. Voucher specimens of the plants were kept at the herbarium of the CPMR.

Pathogenic bacteria used

The 21 bacteria agents used in the study included standard strains of *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 33495), *Pseudomonas aeroginosa* (ATCC 27923), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus saprophyticus* (ATCC 15305), *Proteus mirabilis* (ATCC 49565), *Salmonella typhi* (ATCC 19430), and *Salmonella typhimurium* (ATCC 14028). In addition, identified clinical isolates consisting of two strains each of *E. coli*, *K. pneumoniae*, *P. aeroginosa*, *S. aureus*, *S. saprophyticus*, *P. mirabilis*, *S. typhi*, and *S. typhimurium* were obtained from the Department of Microbiology, Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. These test strains were maintained on nutrient agar (NA) slopes at 4°C and were sub-cultured for use when needed.

Preparation of ethanol extract of “Antibact”

Five hundred grams (500 g) of equal proportions of each of the pulverized plant materials was cold macerated with 70% ethanol for two days (48 h). The ethanol extracts were concentrated using Heidolph rotary evaporator (LABOROTIA 4000, Germany) at a temperature of 65°C. Twenty five millilitres (25 ml) portions of the concentrated ethanol extracts were poured into 250 ml flasks and lyophilized using a Heto Power Dry LL3000 freeze-dryer (Jouan Nordic, R507/200 g, Germany) for 24 h. The freeze-dried powders obtained were also stored in air-tight containers and stored in a refrigerator at 4°C until needed.

Preparation of aqueous extract of Antibact

Aqueous fractions (decoctions) of the plant materials were prepared by boiling 1000 g of equal proportions of the dried plant materials in 2000 ml of clean water for about 45 min. The resultant extracts were concentrated using reduced temperature for another 60 min. The extracts were allowed to cool and subsequently lyophilized as described in the ethanol extracts. The freeze-dried powders obtained were also stored in air-tight containers and stored in a refrigerator at 4°C until needed.

Phytochemical analysis of aqueous and ethanol extracts of “Antibact” used

The phytochemical constituents of the aqueous and ethanol “Antibact” were determined by the protocols described by Krishnaiah et al. (2009). The phytochemical parameters assayed for, included alkaloid, flavonoids, polyuronides, reducing sugars,

cyanogenic glycoside, saponins, triterpenes, anthracenosides, phytosterols and phenols.

Safety of aqueous and ethanol "Antibact" obtained (acute toxicity (LD_{50}) test)

The LD_{50} study of the aqueous and ethanol extracts of "Antibact" was carried out using Sprague-Dawley female rats weighing between 250 and 300 g. The herbal extract was filtered and freeze dried to get the lyophilized extract. Dose levels of 5000, 2500, and 1250 mg/kg of the freeze dried extract were administered orally to the rats per kilogram body weight. The animals were observed for the first 24 h and then a period of 48 h for signs of toxicity such as: effect on eyes (eye colour, tears in eyes, bulging), effect on movement (sluggish movement or immobile), effect on breathing (quick or slow), arrangement of fur (pilo-errection), and twitching gait. General observations other than the aforementioned normal behavior were also observed and recorded. The LD_{50} value was expressed as the weight of extract administered per kilogram body weight of the experimental rats and the values obtained were compared to the Hodge and Sterner toxicity scale (CCOHS, 2005).

Antibiotics sensitivity test

The *in vitro* antibiotic sensitivity test was performed using Kirby-Bauer disc diffusion method as described by National Committee for Clinical Laboratory Standard (NCCLS, 1998). The antibiotics chosen were based on the Clinical and Laboratory Standards Institute (CLSI) recommendations (CLSI, 2007) as well as current treatment regimens for microbial infections in Ghana (MOH, 2010). Briefly, 2 to 6 h cultures of the microbes in peptone water that had achieved 0.5 McFarland standard turbidity were seeded over Muller-Hinton agar by the swabbing technique. Selected antibiotic disc were carefully placed on the surface of the agar and incubated at 37°C for 16 to 18 h. The zones of inhibition of the various antibiotics were measured with a meter rule by taking the diameter of the zones. The measured zones were compared to standard antimicrobial sensitivity chart and recorded as sensitive or resistant to the respective antibiotics. The antibiotics that were tested included: Amikacin (30 µg/disc), Ampicillin (10 µg/disc), Penicillin (10 µg/disc), Cloxacillin (5 µg/disc), Erythromycin (15 µg/disc), Tetracycline (30 µg/disc), Gentamicin (10 µg/disc), Cotrimoxazole (25 µg/disc), Chloramphenicol (30 µg/disc), and some of the newer generation antibiotics including Cefixime (30 µg/disc), Cefuroxime (30 µg/disc), and Cefotaxime (30 µg/disc).

Antibacterial activity of "Antibact"

Antibacterial activity of the aqueous and ethanol "Antibact" was determined by the agar-well diffusion method as described by CLSI (2007). Sixteen hours old overnight broth cultures were sub-cultured for another 2 h and their turbidity adjusted to 0.5 McFarland standards. Muller-Hinton agar plates were seeded with the 2 h old culture using the swabbing technique. A sterilized cock borer with 4 mm internal diameter was used to punch holes in the medium and about 100 µl of 32% w/v (using sterile distilled water and DMSO as diluents for aqueous and ethanol extracts respectively) of "Antibact" dispensed into the respective labelled holes. Disc of standard drugs 30 µg/disc chloramphenicol was used as positive controls, while 20% v/v DMSO and sterile distilled water were used as negative controls. Triplicates of each plate were made and the procedure repeated for the other microbes. The plates were kept in the refrigerator for about 4 h for complete diffusion of the extract and subsequently incubated at 37°C for 48h. After the incubation period, the diameter of each zone of inhibition was measured in millimeters (mm) with a standard ruler. The minimum inhibitory concentration (MIC) of the "Antibact" was determined for each organism as described previously (Eloff, 1998). The minimum bactericidal concentration (MBC) values were deduced from those wells with lowest concentrations at which no growth took place after sub-culturing for 24 h of incubation as described by Nester et al. (2004).

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 16 version software was used to analyze the frequencies and averages for the resistance patterns of the test organisms and the antimicrobial activity of "Antibact".

RESULTS

Phytochemical constituents

The aqueous and ethanol "Antibact" were subjected to phytochemical screening and the results summarized as shown in Table 1. The study revealed the presence of saponins, reducing sugars, phenolics, polyuronides, and triterpenes as the major phyto-constituents of both aqueous and ethanol "Antibact". Alkaloids and flavonoids were however present only in the ethanol "Antibact" whilst phytosterols were only present in the aqueous "Antibact".

Toxicity test (LD_{50})

As shown in Table 2, the LD_{50} values obtained in this study for both aqueous and ethanol "Antibact" were greater than 5000 mg/kg. This suggests that both herbal medicinal products are practically non-toxic according to Hodge and Sterner scale.

Antibiotic sensitivity test

Twenty one strains of pathogenic bacteria were examined for their susceptibility to standard antibiotics. As shown in Figure 1, all the isolates (100%) were found to be resistant to five or more of all the antibiotics used, namely, Ampicillin (AMP), Chloramphenicol (CHL), Tetracycline (TET), Gentamicin (GEN), Amikacin (AMK), Cotrimoxazole (COT), Erythromycin (ERY), Penicillin (PEN), Cefixime (CXM), Cefotaxime (CTX), Cefuroxime CRX) and Cloxacillin (CXC). All the test microbes were found to be resistant to ampicillin, penicillin, cloxacillin and tetracycline but were variedly susceptible or resistant to the rest of the antibiotics used. Thus, all the microbes used for this study were multiple resistant, that is, resistance to 3 or more antibiotics (Figure 1).

Susceptibility of the microbes to aqueous and ethanol "Antibact"

The aqueous "Antibact" inhibited the growth of 3 out of 7

Table 1. Phytochemical components of “Antibact”.

Phytochemical parameter	Aqueous extract of antibact	Ethanol extract of antibact
Saponins	+	+
Reducing sugar	+	+
Cyanogenic glycosides	-	-
Phenolics	+	+
Polyuronides	+	+
Alkaloids	-	+
Anthracenosides	-	-
Flavonoids	-	+
Phytosterols	+	-
Triterpenes	+	+

+ = Present, - = Absent.

Table 2. Acute toxicity test (LD_{50}) of aqueous and ethanol “antibact”.

Aqueous extract						
Species and strain	No. of animals Sex/group	Route of admin.	Formulation and dosage	Time of deaths and period of observation	Approx. lethal dose (LD_{50})	Signs of toxicity
Sprague-Dawley rats	12 females; 3 groups (N=4)	Oral	Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg	No death occurred during the period of observation; 48 h of observation.	>5000 mg/kg body weight	Nil
Ethanol extract						
Species and strain	No. of animals Sex/group	Route of admin.	Formulation and Dosage	Time of deaths and period of observation	Approx. lethal dose (LD_{50})	Signs of toxicity
Sprague-Dawley rats	12 females; 3 groups (N=4)	Oral	Freeze-dried aqueous extract 5000, 2500 and 1250 mg/kg	No death occurred during the period of observation; 48 h of observation.	>5000 mg/kg body weight	Nil

(42.86%) standard strains with zones of inhibition ranging from 9.00 ± 0.00 to 9.67 ± 0.58 mm, while 2 (14.29%) wild strains out of a total of 14 were inhibited with zones of inhibition ranging from 10.00 ± 0.00 to 10.33 ± 0.58 mm. Four (66.67%) of the 6 Gram positive bacteria used in the study were susceptible to the aqueous “Antibact”. However, only 1 (6.67%) out of the 15 Gram-negative bacteria was inhibited in growth by the aqueous “Antibact”. In total, the aqueous “Antibact” inhibited the growth of 5 out of 21 (23.81%) microbes used with an average zone of inhibition of 9.73 ± 0.35 mm (Figure 2). In the case of the ethanol “Antibact”, the growth of 4 out of 7 (57.14%) standard strains were inhibited with zones of inhibition ranging from 9.00 ± 0.00 to 14.00 ± 0.00 mm. Nine (64.29%) out of 14 wild strains were however susceptible to the ethanol “Antibact” with zones of inhibition ranging from 9.00 ± 0.00 to 16.00 ± 1.73 mm. The ethanol “Antibact” inhibited all 6 (100%) Gram-positive bacteria used in the study. It however inhibited 9 (60.00%) of the 15 Gram-negative bacteria used. The ethanol “Antibact” in total inhibited the growth of 13 (61.90%) out of the 21 microbes used with an average inhibition zone of 10.80 ± 0.18 mm (Figure 2).

MICs and MBCs of aqueous and ethanol "Antibact "

Results of the MICs and MBCs of “Antibact” are as shown in Tables 3 and 4. The aqueous “Antibact” exhibited MICs ranging from 0.5 to 16.0 mg/ml for the standard strains whilst the wild strains showed MIC ranged of 4.0 and 32.0 mg/ml (Table 2). In the case of the ethanol “Antibact”, the MICs ranged between 1.0 and 2.0 mg/ml for the standard strains whilst that of the wild strains ranged from 2.0 to 8.0 mg/ml (Table 3). Results from the MBCs showed that the aqueous “Antibact” is bacteriostatic at concentrations < 32 mg/ml while the ethanol “Antibact” demonstrated better bactericidal activity

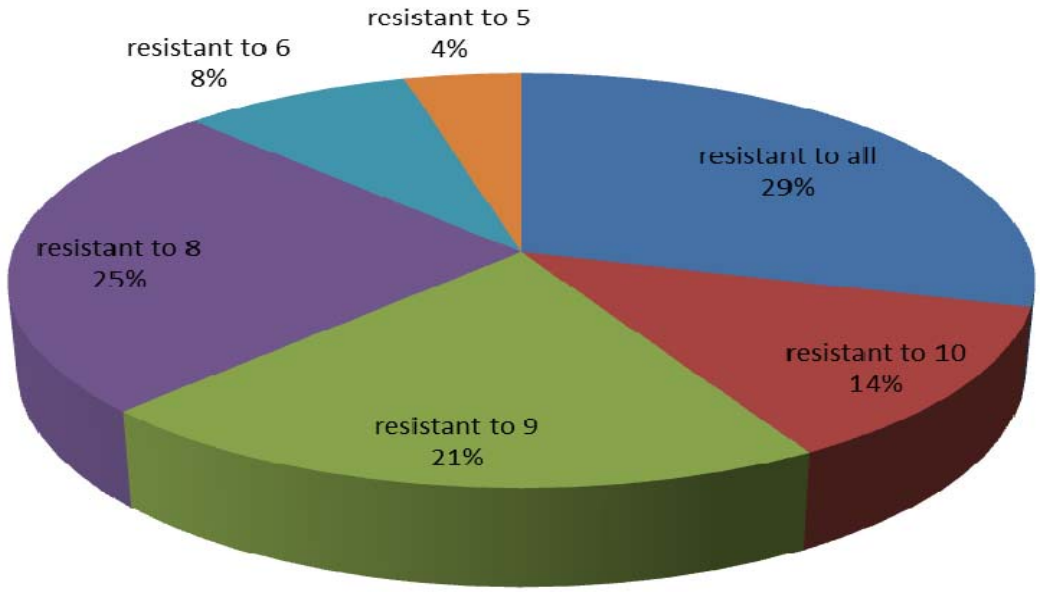

Figure 1. Percentage resistance of the microbes to the antibiotics used.

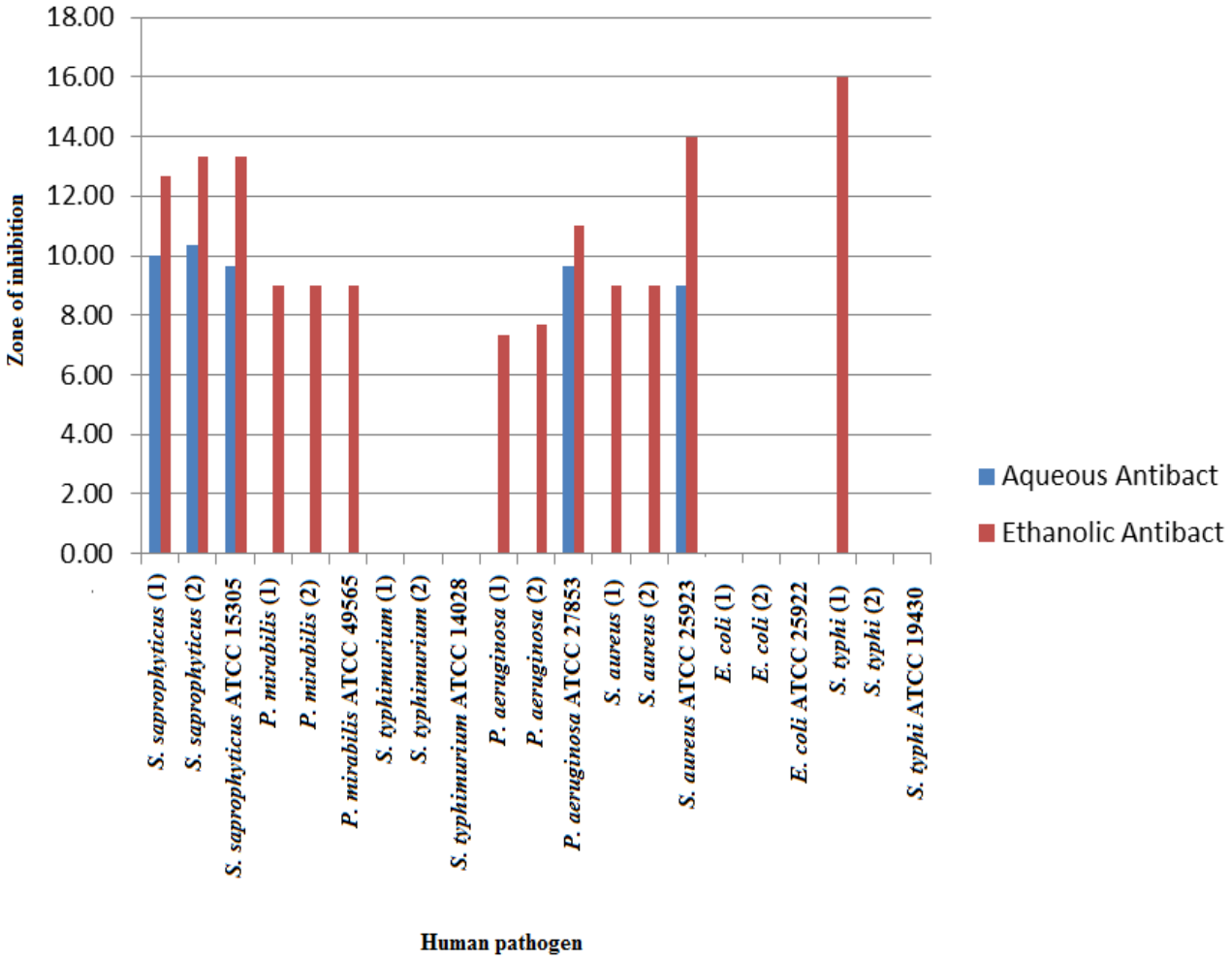

Figure 2. Susceptibility of microbes to the aqueous and ethanol “Antibact”

Table 3. MICs (mg/ml) of the aqueous and ethanol "Antibact".

Pathogenic bacteria	MICs (mg/ml)							
	Aqueous "Antibact"				Ethanolic "Antibact"			
	1	2	3	Average	1	2	3	Average
Wild strains								
S. saprophyticus (1)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
S. saprophyticus (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
P. mirabilis (1)	16.0	16.0	16.0	16.00	4.0	4.0	4.0	4.00
P. mirabilis (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
S. typhimurium (1)	16.0	16.0	16.0	16.00	8.0	8.0	8.0	8.00
S. typhimurium (2)	16.0	16.0	16.0	16.00	4.0	4.0	4.0	4.00
P. aeruginosa (1)	4.0	4.0	4.0	4.00	8.0	8.0	8.0	8.00
P. aeruginosa (2)	32.0	32.0	32.0	32.00	4.0	4.0	4.0	4.00
S. aureus (1)	16.0	16.0	16.0	16.00	8.0	8.0	8.0	8.00
S. aureus (2)	16.0	16.0	16.0	16.00	2.0	2.0	2.0	2.00
E. coli (1)	4.0	4.0	4.0	4.00	4.0	4.0	4.0	4.00
E. coli (2)	32.0	32.0	32.0	32.00	4.0	4.0	4.0	4.00
S. typhi (1)	4.0	4.0	4.0	4.00	4.0	4.0	4.0	4.00
S. typhi (2)	32.0	32.0	32.0	32.00	8.0	8.0	8.0	8.00
Standard strains								
S. saprophyticus ATCC 15305	2.0	2.0	2.0	2.00	2.0	2.0	2.0	2.00
P. mirabilis ATCC 49565	4.0	4.0	4.0	4.00	2.0	2.0	2.0	2.00
S. typhimurium ATCC 14028	8.0	8.0	8.0	8.00	2.0	2.0	2.0	2.00
P. aeruginosa ATCC 27853	8.0	8.0	8.0	8.00	2.0	2.0	2.0	2.00
S. aureus ATCC 25923	16.0	16.0	16.0	16.00	1.0	1.0	1.0	1.00
E. coli ATCC 25922	0.5	0.5	0.5	0.50	2.0	2.0	2.0	2.00
S. typhi ATCC 19430	2.0	2.0	2.0	2.00	2.0	2.0	2.0	2.00

(Table 4).

DISCUSSION

Undoubtedly, infectious diseases are the leading cause of morbidity and mortality globally. The situation has been compounded with the continuous emergence of multi-drug resistant infectious agents, particularly pathogenic bacteria. This phenomenon has led to an increase in investigations into natural products, particularly plant products, as a source of new biomolecules for human disease management (Mohana et al., 2009). Considering the fact that different plants have various medicinal and antimicrobial properties, we put together four medical plants to form two products, aqueous "Antibact" and ethanol "Antibact", potential antimicrobial agents for systemic use. The antimicrobial activities of the products were investigated, and their phyto-constituents and LD_{50} levels established.

The antibiogram of the organisms used showed that none of the microbes examined in this study were susceptible to relatively affordable, commonly prescribed "first-line" antibiotics such as Ampicillin, Penicillin, Tetracycline, and Cloxacillin (Figure 1). In recent times, one of the challenges hampering the smooth treatment of infectious diseases is microbial resistance to anti-microbial agents. For example, beta-lactamase producing bacteria are mostly resistant to beta-lactam drugs such as Penicillins, Cephalosporins, Carbapenems, Monobactams and others (Del Carmen Rodrigue et al., 2004). Quinolones such as norfloxacin, ciprofloxacin, nalidixic acid and others which block bacteria DNA synthesis by inhibiting DNA gyrase (topoisomerase) are now mostly not effective because of mutagens which modify the bacterial DNA gyrase (Baceiro et al., 2013; Fournier et al., 2000). Resistance to Aminoglycosides antibiotics, Tetracycline, Chloramphenicol, Erythromycin, clindamycin and others have been reported (Greenwood et al., 2007).

The emergence of resistant bacterial strains to almost all the "first-line" antibiotics raises public health concerns especially in most developing countries where antibiotics are purchased as over-the-counter drug. Even though there are natural causes of this growing worry of microbial resistance to antimicrobials, the abuse of

Table 4. MBCs of the aqueous and ethanol "Antibact".

Pathogenic bacteria	MBCs (mg/ml)	
	Aqueous "Antibact"	Ethanol "Antibact"
Wild strains		
S. saprophyticus (1)	32.0	8.0
S. saprophyticus (2)	32.0	8.0
P. mirabilis (1)	32.0	4.0
P. mirabilis (2)	32.0	8.0
P. aeruginosa (1)	32.0	8.0
P. aeruginosa (2)	32.0	8.0
S. aureus (1)	32.0	8.0
S. aureus (2)	32.0	8.0
E. coli (1)	32.0	16.0
E. coli (2)	32.0	4.0
S. typhi (1)	32.0	16.0
S. typhi (2)	32.0	16.0
Standard strains		
S. saprophyticus ATCC 15305	32.0	8.0
P. mirabilis ATCC 49565	32.0	4.0
P. aeruginosa ATCC *27853*	32.0	4.0
S. aureus ATCC *25923*	320	8.0
E. coli ATCC *25922*	32.0	4.0
S. typhi ATCC 19430	32.0	4.0

antibiotics by both patients and clinicians, and the widespread use of antimicrobial agents in veterinary medicine are huge contributing factors. In addition to the hunt for new antimicrobials/antibiotics, there should be collective efforts aimed at educating the general public on the safe use of antimicrobial agents.

The results of the phytochemical analysis showed that the herbal medicinal products "Antibact" contain saponins, reducing sugars, phenolics, polyuronides and triterpenes as the major phyto-constituents. Alkaloids and flavonoids were present in only the ethanol "Antibact" while phytosterols were found in the aqueous "Antibact" (Table 1). Terpenoids have been reported to have anti-microbial properties (Scortichini and Pia, 1991). Studies have shown that triterpenes, terpenoids or isoprenoids have relatively high antifungal or antimicrobial properties which affect the non-mevalonate pathway. This pathway is critical for the synthesis of cell membrane components, prenylation of proteins and as a secondary source of carbon for fungi, protozoans, Gram-negative bacteria and other micro-organisms (Nayak et al., 2010). Reducing sugars have been reported to have antibacterial property (Dhale and Markandeya, 2011; Mabeku et al., 2007). Various oils from plants have shown varying degrees of antimicrobial activity (Akgul and Saglikoglu, 2005). The ethanol "Antibact" may contain some oils from the plants which contributed to the enhanced activity it exhibited. In general, the activity of these phytochemical-constituents may be responsible for the antimicrobial activities observed in the study.

Phyllanthus fraternus leaves are reportedly used to treat hepatitis, tuberculosis, viral infections, liver diseases, anemia, dysentry , cystitis, prostatitis, veneral diseases and urinary tract infections (Bapat and Mhapsekar, 2014; Singh et al., 2011). Koffuor and Amoateng (2011) also established in their study that the plant has antioxidant and anticoagulant properties hence confirming its potential in the management of conditions caused by oxidative stress. Study conducted in Kenya re-vealed the use of *Hoslundia opposita* in the treatment of colds, sore throat, gonorrhea, convulsion, stomach pains, and ringworms (Okach et al., 2013). Usman et al. (2010) indicated the plant contains essential oils, and this could be responsible for it broad us in treatment by traditional folks. Crude extract from *Psidium guajava* exhibited similar finding as in the present study (Ofodile et al., 2013; Biswas et al., 2013). Essential oil of *Cymbopongon citrates* has also been reported as potential source of bacteriostatic, fungistatic and microbicide agents against a wide range of infectious organisms (Soares et al., 2013; Vazirian et al., 2012; Lodhia et al., 2009). The antimicrobial activity of the plants confirms their use by

by traditional healers in the treatment and management of some diseases caused by infectious agents.

The LD_{50} test performed on the products revealed that both aqueous and ethanol “Antibact” are safe and non-toxic (Table 2). Our present investigation is the first study indicating the effectiveness of “Antibact”, as significant antimicrobial agent against both Gram negative and Gram positive bacteria (Table 3). The ethanol “Antibact” was significantly effective as compared to the aqueous, inhibiting the growth of 13 out of 21 (62%) microbes used while the aqueous “Antibact” inhibited the growth of 5 out of 21 (23%) microbes used with respective average zones of inhibition of 6.64 ± 1.51 and 8.95 ± 1.42 mm. Several other studies have reported similar observations regarding various solvent systems used in the extraction processes (Bakht et al., 2014; Mills-Robertson et al., 2009). Probably the 70% ethanol has the potential of extracting active ingredients consisting of both polar and non-polar compounds from the product compared to the water which extracts mostly polar compounds. In general, the Gram positive organisms were found to be more susceptible to the “Antibact” than the Gram-negative bacteria used as indicated by previous studies (Biswas et al., 2013; Mills-Robertson et al., 2012).

The porous nature of the cell wall of Gram positive bacteria has been the reason for this observation, as about 90% of the cell wall of Gram-positive bacteria is made of peptidoglycan, which is not a regulatory structure compared to the cell membrane, and therefore, allows most compounds that fit to pass through it. Gram-negative bacteria, on the other hand, have cell wall made of approximately 20% peptidoglycan surrounding a periplasmic space that contains proteins which destroy potentially dangerous foreign matter (Drawz and Bonomo, 2010; Greenwood et al., 2007).

Regardless of the fact that the agar-well diffusion recorded some non-susceptibility by the microbes, the MIC test was performed on all the bacteria strains used in the study. Hundred percent inhibitory activities were seen in all the microbes, suggesting limitations of the agar-well diffusion techniques. It will therefore be appropriate to always use MIC test as the first step when screening medicinal plants for antimicrobial properties. The relatively low MIC values observed (Table 4), especially those exhibited by the ethanol “Antibact” (1.00 to 8.00 mg/ml) give an indication of the effectiveness of the products. Generally, lower MIC values were recorded among the standard strains as compared to the wild strains. Since the wild strains are clinical isolates, it is possible that their exposure to various antibiotics has led to the development of some levels of resistance. The study further revealed that the products have bactericidal properties. However, higher concentrations of aqueous “Antibact” were required to kill the bacteria as compared to that of the ethanol “Antibact”. The observed bactericidal effect of “Antibact” products on the test bacterial isolates is justification for the need to explore the various medicinal plants in order to determine their antimicrobial efficacy and safety.

Conclusion

Conclusively, this study revealed that “Antibact”, herbal medicinal products containing extracts from four plants have antimicrobial properties against selected pathogenic bacteria. However, the ethanol “Antibact” showed better activity than the aqueous “Antibact”. The products are also safe for human use as their LD_{50} values are >5000 mg/kg body weight.

ACKNOWLEDGEMENTS

This study was financially supported by the Centre for Plant Medicine Research (CPMR), Mampong-Akwapim, Ghana. The authors are grateful to the staff of the Department of Microbiology at CPMR, especially Sylvester Kaminta and his colleagues for their support. They also thank the staff of the Plant Development Department for supplying the plants for the study.

Conflict of interests

The authors declare that they have no conflicting interests.

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