

## Full Length Research Paper

## Modulatory effects of *Hillieria latifolia* and *Laportea ovalifolia* on activity of selected antibiotics

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Microorganisms are becoming resistant to almost all existing and newly discovered antimicrobial agents. This has led to ineffective treatment of infectious diseases with increased risk of complications. Few medicinal plants have been found to exhibit the ability of reversing the resistance mechanisms of microbes to antibiotics. The study investigates the influence of leaf methanol extracts of *Hillieria latifolia* and *Laportea ovalifolia* on some commonly used antibiotics. Micro-dilution technique was used to determine the antimicrobial activity and minimum inhibitory concentration (MIC) of the leaf extracts and the selected antibiotics. MICs of the antibiotics in presence of sub-inhibitory concentration of the extracts were determined. MIC of *H. latifolia* and *L. ovalifolia* extracts ranged from 50 to 100 mg/ml. In the presence of sub-inhibitory concentration (5 mg/ml) of the extracts, the activity of the antibiotics was modified with enhanced or reduced activity. The activity of amoxicillin was potentiated by 8-folds, 4-folds, 2-folds, 8-folds, and 2-folds against *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhi*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively in the presence of leaf extract of *H. latifolia*. Activity of ampicillin was potentiated by 2- and 4-folds against *E. coli* and *S. typhi*, respectively, as well as tetracycline, 4-folds, against *Klebsiella pneumonia* in the presence of leaf extract of *H. latifolia*. Sub-inhibitory concentrations of *H. latifolia* and *L. ovalifolia* extracts reduced the activities of erythromycin and ciprofloxacin against all the test organisms. Sub-inhibitory concentrations of *H. latifolia* and *L. ovalifolia* extracts modified the activities of the selected antibiotics.

**Key words:** Minimum inhibitory concentration, antibiotics, microbial resistance, antibiotic-resistance modifying agents.

### INTRODUCTION

Pathogenic organisms which were previously known to have been killed or inhibited by antibiotics are now resistant to these same antimicrobial agents (Levy and Marshall, 2004). This has forced a number of pharmaceutical companies to leave the field of antibiotic

discovery and production to rather produce more profitable medications for treating other diseases especially non-communicable diseases (Projan, 2003). The problem of resistance is now posing great threat on public health more than ever before, due to increasing

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multi-drug resistance in a single organism drastically limiting therapeutic options (Levy, 2005).

Naturally, bacteria have the ability to genetically develop resistance to antibacterial agents (Nascimento et al., 2000). They use different ways and strategies to acquire or develop resistance to antibiotics, which include, active efflux of drugs, alteration of target sites and inactivation of antibiotics by producing enzymes that degrade them (Sibanda and Okoh, 2007). Also, inappropriate diagnosis, drug counterfeit, use of antibiotics in food production and animal rearing, non-compliance and under dosing and sometimes uncontrolled use of antimicrobial agents are factors that contribute greatly to the overwhelming increase in the microbial resistant menace in this generation (Adu et al., 2014).

Microbial resistance to antimicrobial agents has limited the use of known cheap but effective antibiotics (Ranjan et al., 2012). This has necessitated the search for new potent antimicrobial agents to combat the threat posed by resistant microbes. In search of antimicrobial agents, various sources such as the synthetic compounds as well as bioactive agents from natural products (aquatic microorganisms and medicinal plants) are taken into consideration (Agyare et al., 2012). Medicinal plants are great sources of antimicrobial agents and the idea that plants have been used as effective drugs for treatment of infectious diseases was well accepted even before the discovery of microbes (Rios and Recio, 2005).

Coates et al. (2002) reported the occurrence of cross resistance to newly identified antibiotics and other antimicrobial agents suggesting that newly discovered antimicrobial agents may be rendered ineffective in the near future. Even though the appropriate use of these antimicrobial agents can reduce the rate of resistance development, it cannot eliminate the emergence of resistant strains (Sibanda and Okoh, 2007). There is therefore the need to discover and develop new compounds that will target and block resistance mechanisms to help treat infections from these resistant strains (Oluwatuyi et al., 2004).

Medicinal plants have been found to contain compounds with or without antimicrobial property that can cause resistant microorganisms to be susceptible to a previously impotent antibiotic (Aiyegoro et al., 2009). In most developing countries, where majority of the people rely on medicinal plants and natural products, they combine their orthodox medications with these plants in the treatment of various diseases (Adu et al., 2014; Agyare et al., 2009). This study, therefore, investigates the influence of leaf methanol extracts of *Hillieria latifolia* and *Laportea ovalifolia* on some commonly used antibiotics. *H. latifolia* (Lam.) H. Walt belongs to the family Phytolaccaceae and is locally known by the Ewes as 'avegboma' and 'anafranaku' by the Asantes in Ghana. It is a perennial herb of 30 to 120 cm high, with ovate-elliptic leaves and numerous short hair-like

structures on lower surface. The leaves are used in Ghana for the management of rheumatism, boils and wounds (Agyare et al., 2009). The leaves are used to treat general oedema, asthma and some skin diseases (Mshana, 2000; Dokosi, 1998). It is also used to treat cough with blood (Schmelzer and Gurib-Fakim, 2008).

*L. ovalifolia* (Schumach.) Chew belongs to the family Urticaceae. It is known by the Asantes in Ghana as 'akyekyenwonsa', 'abrewa nom taa' or 'Kumasi otuo'. It is a herbaceous weed more often creeping than erect and densely covered with stinging hairs (Chew, 1969). *L. ovalifolia* is of two varieties, that is, male and female (Essiett et al., 2011). Leaves are used to treat wounds (Agyare et al., 2009) and the fruits are used as a poison antidote (Bouch, 2004). The root extract is used to prevent or reduce excessive menstrual bleeding (Sofowora, 1996)..

## MATERIALS AND METHODS

### Plant collection

Leaves of *H. latifolia* and *L. ovalifolia* were collected from Aburi in the Eastern region of Ghana on February, 2014. The plants were authenticated by Dr. Alex Asase of the Department of Botany, University of Ghana, and voucher specimen AA 63 and AA 71, respectively deposited in the Ghana Herbarium, Department of Botany, University of Ghana, Legon, Accra, Ghana.

### Plant extraction

The fresh leaves collected were washed thoroughly under running tap-water and dried under shade between 25 and 28°C for two weeks, after which they were pulverized into coarse powder using the laboratory milling machine (Christy and Norris, Chelmsford, England). 800 g each of the powdered plant materials were soaked in 2.5 L of 70% v/v methanol. They were extracted with the aid of ultra-turrax (T 25 Janke and Kunkel, Laborteknik, Germany) under ice-cooling at a speed of 24000 rpm for 3 to 5 min, and then filtered using a laboratory sieve (Retsch, Haan, Germany) of mesh number 200 with aperture of 75 µm and Whatmann filter paper No.1. The filtrates were concentrated with the rotary evaporator (Rotavapor BÜCHI R-200 with heating bath B-490, Büchi, Konstanz) at 40°C under reduced pressure and lyophilized and then stored in air tight containers at 4 to 8°C. The yields of the extracts of *H. latifolia* and *L. ovalifolia* were 17.4 and 11.29% w/w, related to the dried material, respectively.

### Preliminary phytochemical screening

Methanol leaf extracts of *H. latifolia* (HLE) and *L. ovalifolia* (LOLE) and their respective powdered dried plant materials were subjected to qualitative phytochemical analysis to identify various secondary metabolites such as tannins, glycosides, saponins, alkaloids, flavonoids, steroids and terpenoids present using standard methods described by Usman et al. (2014), Trease and Evans (2002) and Sofowora (1993).

### High performance liquid chromatography (HPLC) profile of extracts

HPLC analysis was performed to identify the profile or finger-prints

**Table 1.** Phytochemical screening of methanol leaf extracts of *H. latifolia* and *L. ovalifolia*, and their dried powdered plant materials.

Secondary metabolites	<i>H. latifolia</i> leaf		<i>L. ovalifolia</i> leaf	
	HLEE	Powdered plant material	LOLE	Powdered plant material
Tannins	+	+	+	+
Flavonoids	+	+	-	-
Glycosides	+	+	+	+
Saponin	+	+	-	+
Alkaloids	+	+	-	-
Sterols	+	+	+	+
Terpenoids	+	+	+	+

(+) = Presence of secondary metabolites; (-) = absence of secondary metabolites. HLEE: Methanol leaf extract of *H. latifolia*; LOLE: methanol leaf extract of *L. ovalifolia*.

of the crude extracts with a UV-detector set at a wavelength of 254 nm. The running conditions included injection volume of 10 µl, mobile phase of methanol:water (20:80 v/v, isocratic condition), flow rate of 1 ml/min and pressure of 15 MPa. The chromatographic data were determined using Chrom Quest® software.

### Test organisms

Test organisms were obtained from the microbiology laboratory of the Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. They include *Pseudomonas aeruginosa* ATCC 4853, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* NCTC 10073, *Enterococcus faecalis* ATCC 29212 and clinical strains of *Streptococcus pyogenes*, *Salmonella typhi* and *Klebsiella pneumoniae*. They were stored in 30% glycerol broth at -4°C in a frost free freezer (Mistral, UK) until needed, whereby 100 µl of the stock suspension was transferred into 10 ml nutrient broth (Oxoid Limited, United Kingdom) and incubated at 37°C for 24 h (sub-cultured) before use.

### Determination of antibacterial activity and minimum inhibitory concentration (MIC) of extracts

Micro-dilution method described by Eloff (1998) and modified by Agyare et al. (2012) was used to determine the antibacterial activity and the MIC of the extracts. Each well of micro-titre plate (96 wells) was filled with 100 µl of double strength nutrient broth, 20 µl of 10<sup>6</sup> cfu/ml of the test organisms and 80 µl of different concentrations of HLEE and LOLE ranging from 1.56 to 100 mg/ml. Ciprofloxacin (Sigma-Aldrich, Michigan, USA) at concentration range of 1.0 to 128 µg/ml was used as reference antibiotic drug. Control wells were filled with broth only and broth and test organisms only. After 24 h of incubation at 37°C, 20 µl of 1.25 mg/ml of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich, Taufkirchen, Germany) was added to each well and observed for a purple colouration after incubation at 37°C for 30 min which indicated microbial growth. The minimum concentrations of HLEE, LOLE and reference drug that did not show any colour change in the wells were recorded as the MIC. The method was replicated three times to validate the results.

### Microbial resistance modifying activity of the extracts

This study was done to determine the effect of a sub-inhibitory

concentration of the extracts on the activity of some selected antibiotics including amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin (Sigma-Aldrich, Michigan, USA). The micro-dilution technique with some modifications as described by Adu et al. (2014) was employed. The MICs of the antibiotics were first determined using concentrations ranging from 1 to 1024 µg/ml. Each of the 96 wells of the micro-titre plate was filled with 100 µl of double strength nutrient broth, appropriate volume of different concentrations of the antibiotics and 20 µl of 10<sup>6</sup> cfu/ml of the test organisms. The plate was incubated for 24 h at 37°C, after which 20 µl MTT was added to the wells and MIC determined as the lowest concentration at which no growth was observed (that is, no colour change from yellow to purple). MICs of the antibiotics were re-determined in the presence of sub-inhibitory concentration (5 mg/ml) of the extracts (HLEE and LOLE).

## RESULTS

Various phytochemical tests were performed on HLEE and LOLE and their dried powdered plant materials to identify their phytochemical composition. Phytochemical screening of HLEE and dried powdered leaf material of *H. latifolia* revealed the presence of tannins, flavonoids, glycosides, saponins, alkaloids, sterols and terpenoids. LOLE and the pulverized leaf material of *L. ovalifolia* also showed the presence of tannins, glycosides, sterols and terpenoids. However, saponins was found in the powdered leaf material of *L. ovalifolia* but was absent in its extract (Table 1).

### HPLC profiles of extracts

Chemical profiles of the extracts indicate the present of metabolites/compounds at the wavelength used and these will serve as a guide in identifying the plants (Figures 1 and 2).

### Antibacterial activity and MIC of extracts

MICs of HLEE and LOLE against typed and clinical strains of organisms, consisting of Gram-positive bacteria

**Table 2.** MIC of methanol leaf extracts of *H. latifolia* and *L. ovalifolia* against test organisms.

Test organisms	MIC of extracts and reference drug		
	HLE (mg/ml)	LOLE (mg/ml)	Cip (µg/ml)
<i>B. subtilis</i>	50.0	50.0	2.0
<i>S. aureus</i>	50.0	100.0	4.0
<i>E. feacalis</i>	50.0	100.0	4.0
<i>S. pyogenes</i>	50.0	100.0	4.0
<i>E. coli</i>	50.0	100.0	2.0
<i>S. typhi</i>	50.0	50.0	4.0
<i>K. pneumoniae</i>	50.0	50.0	4.0
<i>P. aeruginosa</i>	50.0	50.0	4.0

HLE: Methanol leaf extract of *H. latifolia*; LOLE: methanol leaf extract of *L. ovalifolia*; Cip: Ciprofloxacin.

(*B. subtilis*, *S. aureus*, *E. feacalis*, *S. pyogenes*) and Gram-negative bacteria (*E. coli*, *S. typhi*, *K. pneumoniae*, *P. aeruginosa*) were between 50 and 100 mg/ml (Table 2).

#### Antibiotic modulatory activity of methanol leaf extracts of *H. latifolia* (HLE) and *L. ovalifolia* (LOLE)

Activities of the selected antibiotics (amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin) against the test microorganisms were modified in the presence of sub-inhibitory concentration (5 mg/ml) of the extracts, either by enhanced or reduced activity. For instance, the activity of amoxicillin against *E. coli*, *B. subtilis*, *S. typhi*, *S. aureus* and *P. aeruginosa* potentiated by 8-, 4-, 2-, 8- and 2-folds, respectively, in the presence of the sub-inhibitory concentration of HLE. Sub-inhibitory concentration of HLE again enhanced the activity of ampicillin 2- and 4-folds against *E. coli* and *S. typhi*, respectively, as well as tetracycline, 4 folds, against *K. pneumoniae*. The activity of amoxicillin against *S. aureus* and *E. coli* was enhanced (2-folds) in the presence of LOLE. However, both HLE and LOLE sub-inhibitory concentrations reduced the activities of erythromycin and ciprofloxacin against all test organisms (Table 3).

#### DISCUSSION

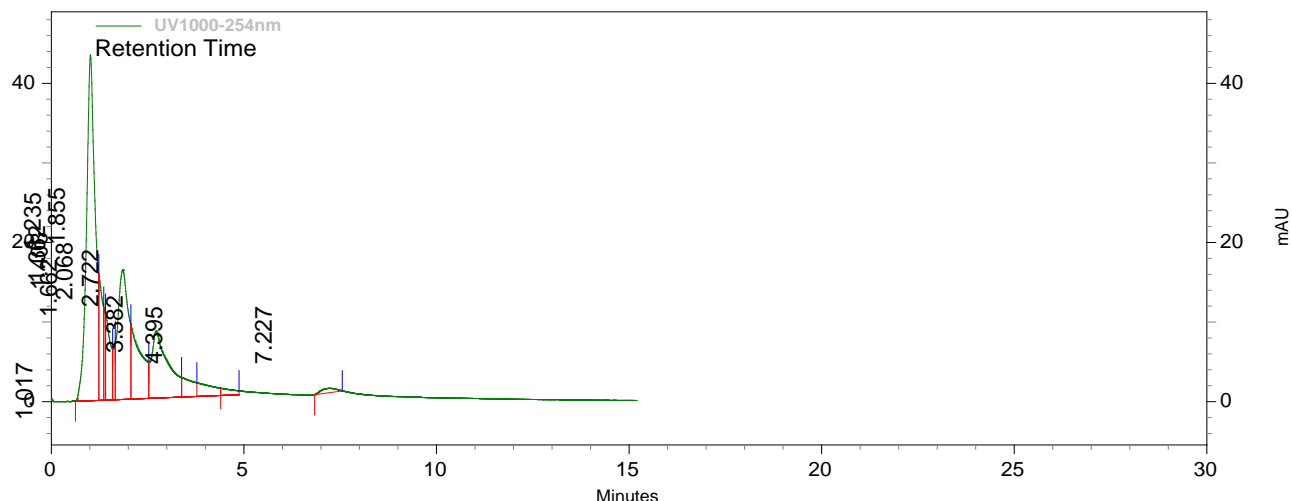
Secondary metabolites such as tannins, flavonoids, glycosides, alkaloids, terpenoids and steroids present in the plants may be responsible for their pharmacological and biological properties (Maganha et al., 2010; Barbosa-Filho et al., 2006; Mbagwu et al., 2007; Sofowora, 1993). These secondary metabolites act alone or in synergy leading to the healing potentials of plants (Jenke-Kodama et al., 2008; Gurib-Fakim, 2006). Phytochemical screening of plant materials revealed the presence of tannins, glycosides, saponins, flavonoids, alkaloids,

sterols and terpenoids in the leaves of *H. latifolia*, which is similar to reports on the same plant by Abotsi et al. (2012) and Schmelzer and Gurib-Fakim (2008). Tannins, glycosides, sterols and terpenoids were also present in the leaves of *L. ovalifolia*, however, flavonoids and alkaloids were absent (Table 1). Essiett et al. (2011) reported the presence of phytochemicals such as tannins, glycosides, saponins, flavonoids and alkaloids in the leaves of *L. ovalifolia*. The absence of flavonoids and alkaloids in the leaves of *L. ovalifolia* as observed in this study may be due to the different geographical location of the plant, the season and time of collection which are all contributing factors leading to variations in the phytochemical constituents of plants of the same species (Stackpole et al., 2011; González-Martínez et al., 2006).

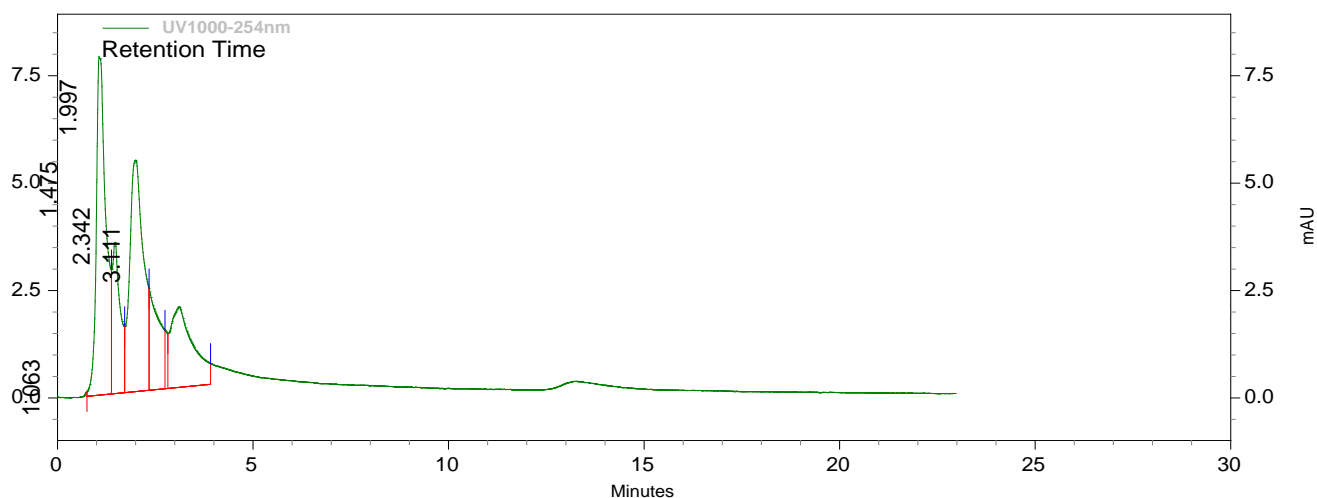
In addition to the phytochemical screening, HPLC profile of the 70% methanol extracts (HLE, and LOLE) were developed for identification purposes. HPLC profiling is more specific and helps in easy identification and confirmation of plant on the basis of specific phytochemicals present. The HPLC profiles of the extracts (Figures 1 and 2) showed that the peaks representing compounds present in the extracts appeared in the early part (early elution) of the chromatogram. This observation may be due to the polar solvent (70% methanol) used for the extraction. The profiles indicate the complex chemical composition of the extracts and provide identification parameters to figure out alterations in crude extracts (Tistaert et al., 2012).

HLE and LOLE exhibited a broad spectrum antibacterial activity against *B. subtilis*, *S. aureus*, *E. feacalis*, *S. pyogenes*, *E. coli*, *S. typhi*, *K. pneumoniae* and *P. aeruginosa* with MIC ranging from 50 to 100 mg/ml (Table 2). The antibacterial activity observed may be attributed to the phytochemical constituents present in the extracts, since these phyto-constituents have been reported to exhibit antimicrobial properties (Edeoga et al., 2005; Nweze et al., 2004).

The low antibacterial activity of HLE and LOLE may be as a result of low amount of the active constituents



**Figure 1.** HPLC profile of methanol leaf extract of *H. latifolia* (HLE) at  $\lambda$  254 nm.



**Figure 2.** HPLC profile of methanol leaf extract of *L. ovalifolia* (LOLE) at  $\lambda$  254 nm.

in the extracts. Similar to this observation, Okwulehie and Akanwa (2013) reported a low antimicrobial activity of *L. ovalifolia*. At MIC of 50 mg/ml, *L. ovalifolia* did not inhibit the growth of the test organisms. Also, Assob et al. (2011) reported on the antimicrobial activity of *H. latifolia* with MIC of 0.6 to 2.5 mg/ml. The high MIC (50 to 100 mg/ml) observed for *H. latifolia* in this study may be as a result of different extraction procedures used and different locality of the plant materials used which may lead to different composition in terms of primary and secondary metabolites.

Even though, HLE and LOLE may not be potential source of antimicrobial agents as reported by Navarro and Delgado (1999) and Fabry et al. (1998) because of their relatively high MICs, it is also well noted that

plant extracts with low antimicrobial activity may have some phyto-constituents that can modify the antimicrobial activity of some existing antimicrobial agents, especially against resistant bacteria (Adu et al., 2009). Sub-inhibitory concentrations (5 mg/ml) of HLE and LOLE modified the activity of amoxicillin, erythromycin, ciprofloxacin, tetracycline and ampicillin by either potentiating or reducing their activity against the test organisms.

The increased activity of these antibiotics in the presence of the sub-inhibitory concentration of the extracts may be attributed to the phytochemicals present in the extracts. For instance, flavonoids have been reported to have the ability to reverse the resistance of *S. aureus* to some antibiotics (Aiyegoro et al., 2009). The

**Table 3.** Effect of sub-inhibitory concentration (5mg/mL) of HLE and LOLE on activity of selected antibiotics against test organisms.

Antibiotic only/Antibiotic + HLE/ LOLE		Test organisms							
		BS	SA	EF	SP	EC	ST	KP	PA
		Number of folds increase in activity							
Amx	HLE	4	8	1	>0.25	8	2	1	2
	LOLE	1	2	1	>0.25	2	0.5	0.5	1
Amp	HLE	1	1	1	>0.0625	2	4	1	1
	LOLE	0.25	1	1	>0.0625	1	0.25	0.5	>0.0625
Tet	HLE	1	1	1	1	0.5	1	4	2
	LOLE	0.125	0.25	0.25	0.25	0.125	0.25	0.5	0.5
Ery	HLE	0.25	0.0625	0.125	0.125	0.0625	0.0625	0.125	0.25
	LOLE	0.5	0.125	0.25	0.0625	0.0625	0.125	0.125	0.125
Cip	HLE	0.125	0.5	0.5	0.5	0.125	0.25	0.5	0.25
	LOLE	0.0625	0.125	0.25	0.25	0.125	0.125	0.125	0.125

EC: *E. coli*, BS: *B. subtilis*, ST: *S. typhi*, KP: *K. pneumonia*, SA: *S. aureus*, EF: *E. feacalis*, SP: *S. pyogenes*, PA: *P. aeruginosa*. Amx: amoxicillin, Amp: ampicillin, Tet: tetracycline, Ery: erythromycin, Cip: ciprofloxacin, HLE: methanol leaf extract of *Hillieria latifolia*, LOLE: methanol leaf extract of *Laportea ovalifolia*.

activity of the test antibiotics was enhanced mainly by HLE as compared to LOLE. This may be as a result of the presence of flavonoids in HLE and its absence in LOLE. Antimicrobials from plants, at sub-inhibitory concentrations, have been reported to be efficient in synergism with antibiotics by enhancing their antimicrobial actions (Kamatou et al., 2006). The phytochemicals act by reversing the resistance mechanisms of some microorganisms, thereby rendering them susceptible to previously inactive antibiotics (Tenover, 2006). Plants have also been known to produce multi-drug resistance inhibitors (MDRIs) to enhance the antimicrobial activities of compounds (Stermitz et al., 2000).

The reduced or nullified activity of the antibiotics may be as a result of interactions between the phytochemicals in the extract and the antibiotics or the microorganisms. The phyto-constituents may react chemically with the antibiotics leading to loss of activity (Adu et al., 2009, 2014). It has been established that certain substances including plant constituents can shield microorganisms from the lethal effects of some antimicrobial agents (Keweloh et al., 1989). For example, some phytochemicals can bind to the surface structures of microorganisms thereby reducing their permeability to antibiotics (Adu et al., 2014). Furthermore, some of the phyto-constituents may act as protein activators or co-enzymes which bind to and activate enzymes responsible for resistance in an organism, making them resistant to a previously potent antibiotic (Lambert, 2002). There is need to isolate the bioactive agents or compounds

responsible for the antibiotic resistance modifying properties especially those that potentiated the activity of the extracts against resistant bacterial strains.

## Conclusion

The sub-inhibitory concentrations of the methanol leaf extracts of *H. latifolia* and *L. ovalifolia* modified the activity of some antibiotics by either potentiating or reducing their antibacterial activities.

## Conflict of interests

The authors have declare no conflict of interests.

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