

Aqueous Extracts of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. Inhibited *Helicobacter Pylori*

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Abstract

The inhibitory effects of methanol, dichloromethane and aqueous extracts of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. (family Combretaceae) reportedly used in the treatment of gastrointestinal diseases including stomach disorder and ulcer are investigated to ascertain and justify their use in traditional medicine. Nineteen (19) strains of *Helicobacter pylori* including 18 clinical isolates and *Helicobacter pylori* ATCC 43504 were used in this study. The susceptibility and minimum inhibitory concentration (MIC) testing were performed using the agar dilution procedure guidelines of the Clinical and Laboratory Standards Institute. bactericidal studies were performed using viable counting techniques. The MIC and MBC values for the susceptible strains ranged from 0.08 mg/mL to 1.25 mg/mL and 0.16 mg/mL - 2.5 mg/mL respectively. Bactericidal study revealed a dose- and time-dependent decline in surviving population of *H. pylori* AB005 at concentrations equivalent to MIC, 2 x MIC and 4 x MIC (Figures 1-4). The aqueous extracts of *Anogeissus leiocarpus* stem bark and root gave a 100% kill at 6-hour exposure time with concentration equivalent to 4x MIC (i.e. 0.32mg/mL). The antimicrobial activities demonstrated by extracts of the plants on *Helicobacter pylori* revealed the presence of therapeutically potent antibacterial compounds and thus justify the use of these medicinal plants for the treatment of peptic ulcer disease.

Keywords: *Anogeissus leiocarpus* (DC.) Guill. & Perr.; *Terminalia glaucescens* Planch ex Benth.; *Helicobacter pylori*; peptic ulcer disease; bactericidal activity

1. Introduction

The use of medicinal plants for the cure of various diseases is very wide spread in many parts of the world including Nigeria among the rural population who cannot afford modern medicines which most often is beyond their reach. This practice involves the use of various plants/plant parts in different part of the world to treat human diseases and infections. Infection with *Helicobacter pylori* the aetiologic agent of gastritis, peptic ulcer disease (PUD), gastric MALT lymphoma and gastric cancer can be effectively treated with medicinal plants. Various medicinal plants have shown activity against *Helicobacter pylori* by successfully inhibiting its growth and multiplication in *in vitro* studies (Lawal *et al.*, 2014a; Olorunnipa *et al.*, 2013; Adeniyi *et al.*, 2009; Mahady *et al.*, 2005; 2003). *In vivo* studies have also been reported with significant reduction in bacterial load as well as inflammation in *H. pylori*-infected experimental animal models (Xie *et al.*, 2013; Gaus *et al.*, 2009). In our earlier study, we reported the anti-*Helicobacter pylori* activities of dichloromethane and methanol extracts of *Myristica fragrans* Houtt. seed (nutmeg) where culture and histopathological examinations revealed a clear reduction in the *H. pylori* colonization and mucosal inflammation and an increase in epithelial proliferation in the stomach of *H. pylori*-infected rats (Oyedemi *et al.*, 2014).

In this study, the inhibitory effects of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. (family Combretaceae) reportedly used in the treatment of gastrointestinal diseases including stomach disorder and ulcer are investigated to ascertain and justify their use in traditional medicine. *Anogeissus leiocarpus* (DC.) Guill. & Perr. is traditionally acclaimed to be effective in treating infectious wounds in man and animals (Dweek, 1996). The infusion and decoctions are used as cough medicine, the pulped roots are applied to wounds and ulcers (Ibrahim *et al.*, 2005). In Côte d'Ivoire the fleshy roots are used against labour pains and in Burkina Faso to accelerate wound healing. The seeds have a wide bactericidal and fungicidal activity in humans and animals (Yahaya *et al.*, 2008).

The decoction of young leaves and the bark of *Terminalia glaucescens* are used for the treatment of stomach ache and abdominal pains. It is a valuable plant in the management of malaria; diarrhoea and tooth decay (Ojo *et al.*, 2006). The leaf and bark find application in the treatment of naso-pharyngeal infections. The roots are used as medicines for the management of diarrhoea, dysentery, genital stimulants, depressants, leprosy and liver disorder (Burkill, 1985). The root bark enters a treatment for burns and for sores in general in Ivory Coast. In Ubangi-Shari root-bark is used on wounds and is said to have an effect like tincture of iodine (Mangaret *et al.*, 1988).

2. Materials and Methods

2.1 Plant collection, extraction and preparation of extracts

Anogeissus leiocarpus (leaf, stem bark and root) and *Terminalia glaucescens* (root) plant materials were collected, identified with voucher specimens assigned FHI 109925 and FHI 108282 for *Anogeissus leiocarpus* and *Terminalia glaucescens*, respectively by the Forest Research Institute of Nigeria Ibadan, Oyo State (FRIN). Plant materials were extracted by cold maceration in methanol, dichloromethane and water. Methanol, dichloromethane and aqueous extracts of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. were reconstituted in 0.5% DMSO to final concentrations at 20, 10, 2 and 1 mg/mL. Lower concentrations in the range of 2.5 to 0.02 mg/mL were also prepared to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of the bioactive extracts.

2.2 Bacterial Isolates

Nineteen (19) strains of *Helicobacter pylori* including 18 clinical isolates and *Helicobacter pylori* ATCC 43504 were used in this study. The clinical isolates were *H. pylori* AB003, 005, 010, 012, 015, 018, 020, 021, 024, 025, 026, 032, 033, 036, 037, 038, 042 and *H. pylori* AB044. The test organisms were subcultured from stored slopes at the Department of Pharmaceutical Microbiology Laboratory.

2.3 Susceptibility Testing and Determination of Minimum Inhibitory Concentration

The susceptibility and minimum inhibitory concentration (MIC) testing were performed using the agar dilution procedure guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2008). To 19 mL of sterile molten Mueller-Hinton agar supplemented with 5% defibrinated horse blood was added 1 mL of the extract. The final concentrations of the extracts tested were 20, 10, 2 and 1 mg/mL reconstituted with 0.5% DMSO. MacFarland 2 suspensions of *H. pylori* strains were prepared in Tryptic soy broth from 4–5 days old *H. pylori* on blood agar. The organisms were inoculated onto agar plates containing the plant extracts via a graduated inoculating device which delivers inoculum size of 1×10^4 cfu/mL per spot. The spots were air-dried before incubating the plates at 37°C under a microaerophilic gas mixture composed of 10% CO₂, 5% O₂, and 85% N₂ (Campygen, Oxoid, UK) at 100% humidity and examined for growth after 72 hours. Ciprofloxacin at 10 µg/mL and 20 µg/mL was used as positive control. All procedures were repeated for accuracy. The least concentration that gave no visible growth was taken as the minimum inhibitory concentration (MIC) of the extract.

2.4 Determination of Minimum Bactericidal Concentration

The minimum bactericidal concentration (MBC) testing was performed as previously described (Lawal *et al.*, 2014b). The lowest concentration that prevented bacterial growth after days of incubation was recorded as the minimum bactericidal concentration (MBC). All tests were performed in duplicates to ensure accuracy.

2.5 Bactericidal Kinetics

The bactericidal activity was performed using viable counting techniques previously described (Ogudo *et al.*, 2014). The concentrations of the extracts used for the kill kinetics were those corresponding to MIC, 2 x MIC and 4 x MIC for the aqueous extracts. Samples were withdrawn at zero minute (0 min), 0.5, 1, 2, 4, 6 hour and 24 hours. The inoculum was evenly dispersed using sterile glass spreader. The drug control and solvent control ran simultaneously with the test. The plates were appropriately incubated for 24 hours after which the number of colonies on each plates were counted using Stuart scientific colony-counter. The number of survivors (cfu/mL) was calculated taking into consideration the final volume of inoculum plated out and the dilution factor. The results are the average of duplicate tests. A graph of viable count (Log₁₀) against time (hour) was plotted to show the rate of kill of the test organism.

3. Results and Discussion

The use of medicinal plants in the treatment of gastrointestinal diseases including peptic ulcer diseases (PUD) in which *Helicobacter pylori* is implicated is becoming widespread due to the difficulty in the treatment choice of *Helicobacter pylori* infection. Treatment choice involving triple or quadruple combination of drugs do not often effect cure of the disease because healing of PUD is hinged on eradication of *H. pylori*. Thus, it has become increasingly necessary to look in the direction of alternative therapy involving natural remedies especially medicinal plants for the cure of *H. pylori* infections and resulting diseases. Hence, *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. (family Combretaceae) identified from our ethnobotanical survey of plants used for the treatment of gastrointestinal diseases were evaluated for their inhibitory effects on *Helicobacter pylori*. Nineteen isolates of *H. pylori* were screened in the study out of which sixteen including *H. pylori* ATCC 43504 (CagA+ strain and a carcinogen) were susceptible to the methanol and aqueous extracts of the test plants with diameter of zone of inhibition ranging between 10 ± 0.0 mm to 29 ± 0.0 mm (data not shown). The dichloromethane had little/no activity. Bioactive extracts were further tested to

determine the minimum inhibitory concentration (MIC), minimum bactericidal concentration as well as kill kinetics. The inhibition of *H. pylori* was dose-dependent as revealed by the diameter of zone of inhibition which at the highest concentration of 20 mg/mL compared favourably with clarithromycin (pure drug control) at 10 µg/mL. The MIC and MBC values for the susceptible strains ranged from 0.08 mg/mL to 1.25 mg/mL and 0.16 mg/mL - 2.5 mg/mL respectively (Table 1). Bactericidal study revealed a dose- and time-dependent decline in surviving population when *H. pylori* AB005 was exposed to the aqueous extracts of both plants at concentrations equivalent to MIC, 2 x MIC and 4 x MIC (Figures 1-4). The aqueous extracts of *Anogeissus leiocarpus* stem bark and root gave a 100% kill at 6-hour exposure time with concentration equivalent to 4x MIC (i.e. 0.32mg/mL).

Although the mechanism(s) of action the aqueous extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* on *H. pylori* is/are not yet known, it is not unusual for the components of the extracts to act synergistically or additively to exert inhibitory effects on the test organisms. The phytochemical components found in these plants from our phytochemical screening were saponins, flavonoids, tannins, alkaloids, reducing sugar, glycosides and resins all of which are known to possess antimicrobial activities on a wide variety of microorganisms. This is similar to the report of Olajire and Azeez (2011). Terpenoidal fractions isolated from both *Anogeissus leiocarpus* (DC.) Guill. and Perr. (Stem) and *Terminalia avicennioides* Planch. (Root) were reported to possess antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* (Ibrahim *et al.*, 2005). The leaves of *Anogeissus leiocarpus* contain ellagic, gallic and gentisic acids, derivatives of gallic and ellagic acid and several flavonoids (derivatives of quercetin and kaempferol). The high concentration (up to 17%, based on dry matter) of hydrolysable tannins (gallic and ellagic acid derivatives) explains the medicinal usefulness of *Anogeissus leiocarpus* (Kubmarawa *et al.*, 2007). The leaf, stem and the root bark of *Anogeissus leiocarpus* have been reported to contain secondary metabolites such as alkaloids, saponins, flavonoids and glycosides; sterols and phenols were also isolated from its root bark (Mann *et al.*, 2008). *Terminalia* species are known to contain several triterpenes, ellagic acid and derivatives of ellagic acid with broad spectrum antimicrobial activities (Dermarderosian, 2002). The inhibitory activities of *Anogeissus leiocarpus* and *Terminalia glaucescens* on *Helicobacter pylori* as revealed from our study compared fairly well with clarithromycin (a pure drug) and may well position these plants for further research to isolate and characterize the anti-*Helicobacter pylori* compound(s) which can be developed and/or modified to elicit better activity than the existing antibacterial agents currently used for the treatment of peptic ulcer disease. This is perhaps the first report on the anti-*Helicobacter pylori* effects of these plants.

4. Conclusion

The study investigated the antibacterial activity of *Anogeissus leiocarpus* (DC.) Guill. & Perr. and *Terminalia glaucescens* Planch ex Benth. (family Combretaceae) on nineteen strains of *Helicobacter pylori* including *H. pylori* ATCC 43504. The aqueous extracts of both plants were the most active on the test organisms supporting the use of these plants in folklore medicine where they are most often infused in hot water to administer to the patients for the treatment of various diseases including peptic ulcer disease. Although the mechanism of the inhibitory effects observed in these plants have not been studied, further research will focus on the mechanism of inhibitory activity as well as the anticancer effects of these plants on gastric cancer in which *H. pylori* has been implicated.

Table 1: Minimum inhibitory concentration (MIC) (mg/mL) and minimum bactericidal concentration (MBC) (mg/mL) of aqueous extracts of *Anogeissus leiocarpus* and *Terminalia glaucescens* root on susceptible *Helicobacter pylori* strains

Plant Sample	<i>Anogeissus leiocarpus</i> Leaf		<i>Anogeissus leiocarpus</i> Stem bark		<i>Anogeissus leiocarpus</i> Root		<i>Terminalia glaucescens</i> root		Ciprofloxacin (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>H. pylori</i> AB 005	0.625	1.25	0.08	0.16	0.08	0.625	0.08	0.16	10	10
<i>H. pylori</i> AB 010	0.08	0.625	0.16	1.25	0.08	0.625	0.32	0.32	5	10
<i>H. pylori</i> AB 012	0.08	0.16	0.08	1.25	0.08	1.25	0.32	0.625	10	10
<i>H. pylori</i> AB 018	0.16	0.32	0.08	0.16	0.08	0.08	0.08	0.16	5	10
<i>H. pylori</i> AB 020	0.625	0.625	1.25	2.5	0.32	0.625	0.625	0.32	5	10
<i>H. pylori</i> AB 021	0.08	0.16	0.08	0.625	0.08	0.32	0.32	0.16	5	10
<i>H. pylori</i> AB 024	0.08	0.16	0.08	1.25	0.16	0.32	0.32	0.32	5	10
<i>H. pylori</i> AB 026	0.16	0.32	0.16	0.32	0.16	0.32	0.32	0.625	5	5
<i>H. pylori</i> AB 036	0.16	0.32	0.08	0.16	0.08	0.08	0.08	0.16	5	5
<i>H. pylori</i> AB 038	0.08	0.16	0.08	0.32	0.08	0.32	0.32	0.32	5	10
<i>H. pylori</i> AB 042	1.25	0.16	0.32	0.16	0.625	1.25	1.25	0.16	10	20
<i>H. pylori</i> ATTC 43504	1.25	2.5	0.32	0.16	0.32	0.625	1.25	0.08	5	10

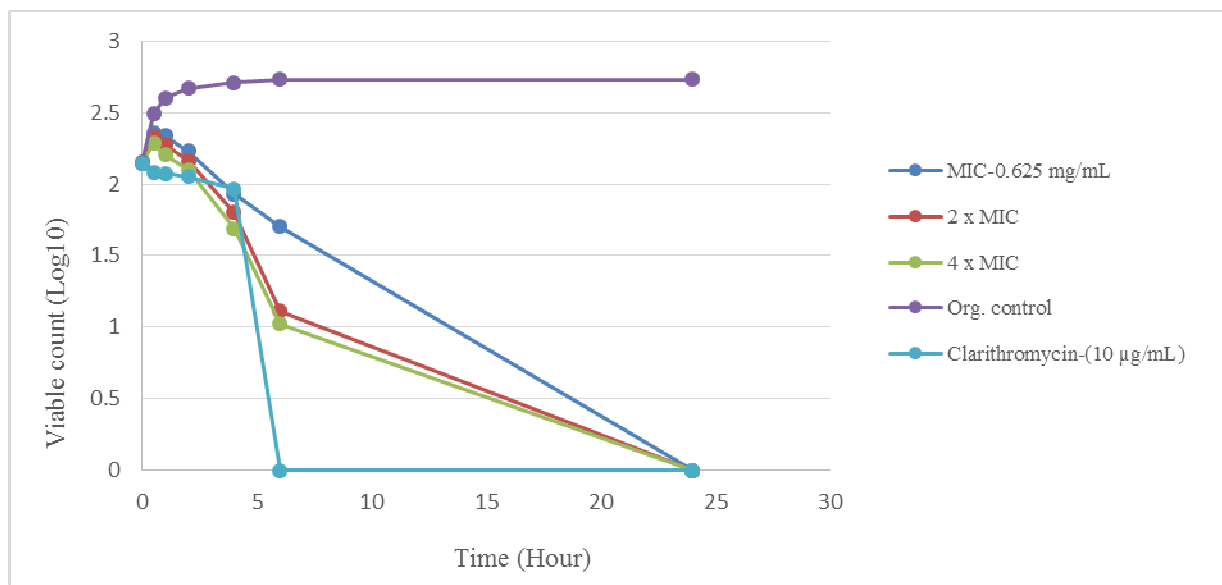


Figure 1: Plot of Viable count (Log₁₀) against Time (hour) of aqueous extract of *Anogeissus leiocarpus* leaf against *Helicobacter pylori* AB 005 showing the rate of kill at the different concentrations and exposure time.

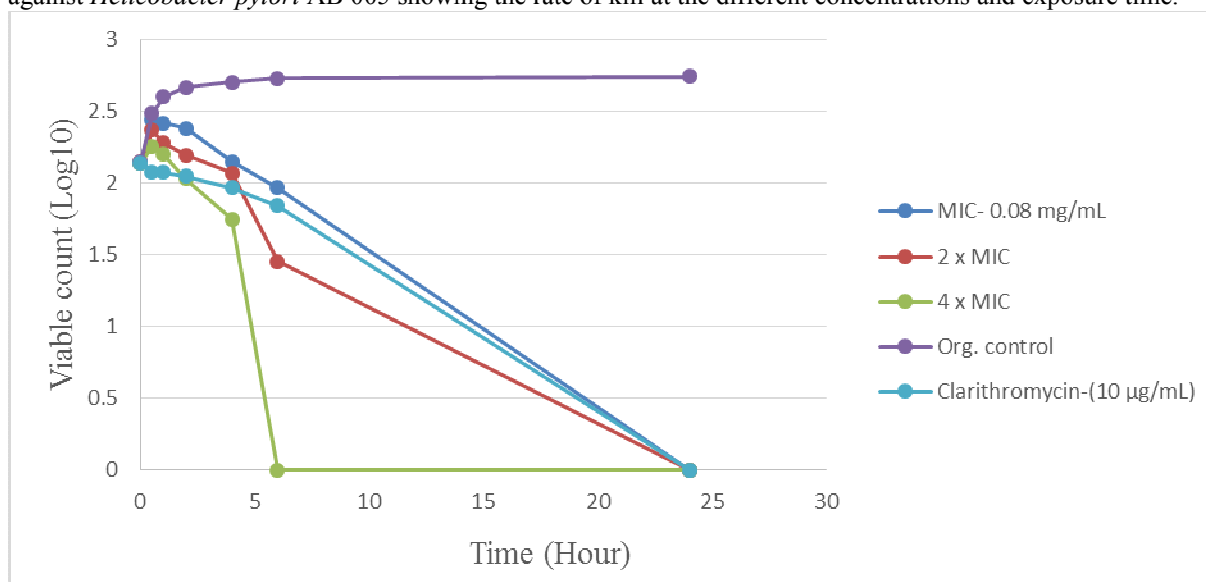


Figure 2: Plot of Viable count (Log₁₀) against Time (hour) of aqueous extract of *Anogeissus leiocarpus* stem bark against *Helicobacter pylori* AB 005 showing the rate of kill at the different concentrations and exposure time.

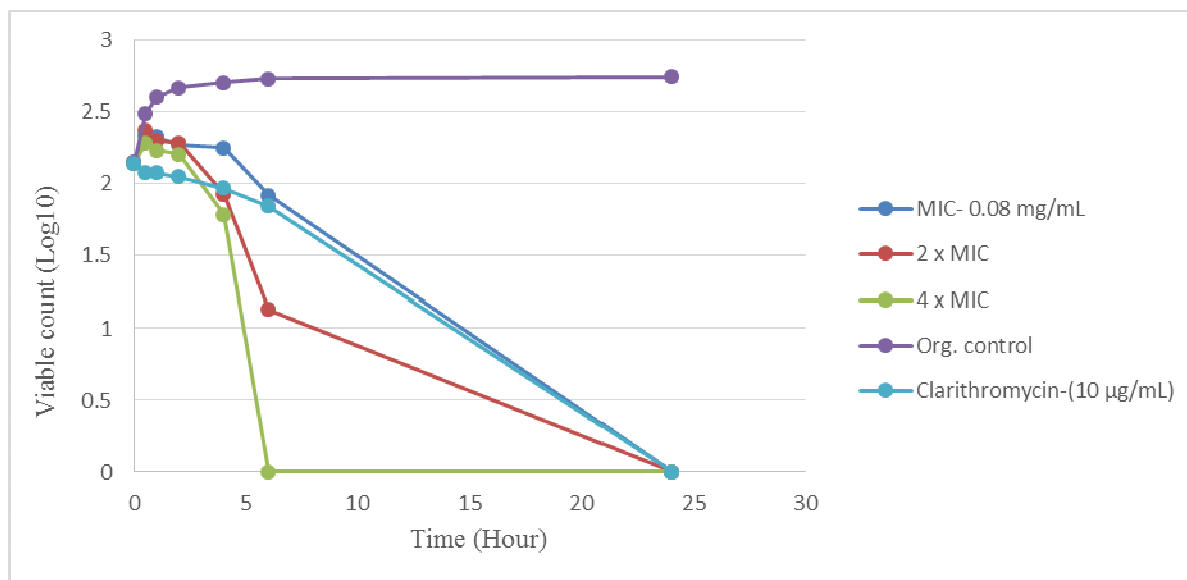


Figure 3: Plot of Viable count (Log10) against Time (hour) of aqueous extract of *Anogeissus leiocarpus* root against *Helicobacter pylori* AB 005 showing the rate of kill at the different concentrations and exposure time.

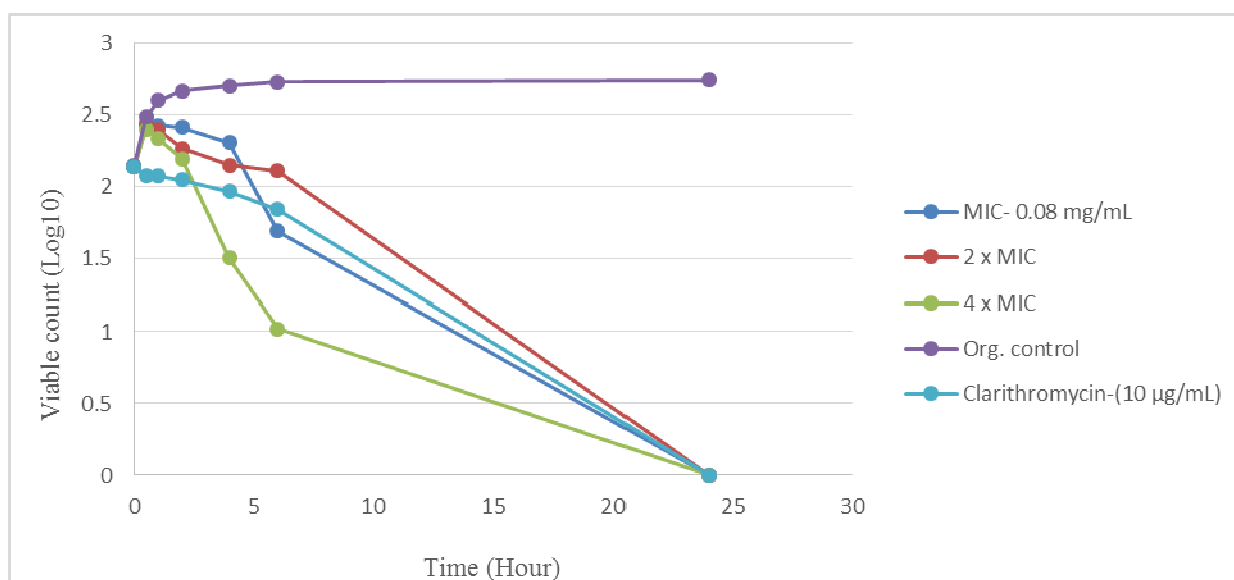


Figure 4: Plot of Viable count (Log10) against Time (hour) of aqueous extract of *Terminalia glaucescens* root against *Helicobacter pylori* AB 005 showing the rate of kill at the different concentrations and exposure time.

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