Journal of Advances in Microbiology
2(4): 1-13, 2017; Article no.JAMB.32277

SCIENCEDOMAIN *international*
www.sciencedomain.org

In-vitro Anti-Bacterial Activity of Extracts of *Euphorbia abyssinica* (Desert Candle) Stem-Bark and Latex

Jacqueline Ebob Tarh[1*] and Christian Ukwuoma Iroegbu[1]

[1]Department of Biological Sciences, Cross River University of Technology, Calabar, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author JET designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript and managed the analyses of the study. Author CUI managed the literature searches and edited the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2017/32277
Editor(s):
(1) Gyanendra Singh, Gene Therapy and Louisiana Vaccine Center, School of Medicine, LSU Health Sciences Center, Louisiana, USA.
Reviewers:
(1) Tanveer Hussain, Mirpur University of Science and Technology (MUST), Bhimber Campus, Pakistan.
(2) Mehlika Benli, Ankara University, Turkey.
(3) Daniela Hanganu, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Romania.
(4) Vinicius V. Pereira, Federal University of Minas Gerais, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/18862

Original Research Article

Received 18th February 2017
Accepted 19th April 2017
Published 1st May 2017

ABSTRACT

Aim: This study was aimed at evaluating *Euphorbia abyssinica* (Desert Candle), a medicinal plant extensively used in folklore medicine among the Kendem people of South-west Cameroon for antibacterial activity and extracts analyzed for phytochemical composition.
Study Design: The completely randomized block design was used and data analyzed using of two way analysis of variance. Significant means were separated using Duncan's New Multiple Range Test.
Place and Duration of Study: This study was carried out in the Department of Microbiology, University of Nigeria Nsukka, Enugu State, Nigeria, between April 2011 and August 2012.
Methodology: Extraction was done using absolute methanol, 50% methanol (in water) and water as solvents. Qualitative analysis methods were used to assay the phytochemical constituents. Agar-well diffusion, macro broth dilution and agar dilution and time-kill assay were the susceptibility test methods adapted.

**Corresponding author: E-mail: j.ebobtarh@yahoo.com;*

Results: The phytochemical constituents detected were alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates and steroids, and saponins. The 50% methanol extract of the stem-bark was highly active against *Staphyloccocus aureus, Escherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa* and compared favorably with the Gentamycin control drug. The inhibition zone diameters (IZDs) obtained with 50% methanol extract measured 23 mm for *S. aureus* and 19 mm for *P. aeruginosa* compared to 18 mm achieved with the absolute methanol extract for both *S. aureus* and *P. aeniginosa.* For the aqueous extract the overall IZD range of 10±1.60-13±2.16 mm. The susceptibility patterns obtained using both dilutions (agar and macro-broth) methods were similar to that obtained with the agar diffusion method above. *S. aureus* (with MIC, 10.93±1.00-; MBC, 25-mg/mL, agar dilution or MIC, 3.9±1.60 -, MBC, 12.5-mg/mL, macro broth dilution methods, respectively). It was considered to be the most significantly susceptible bacteria strain tested (significant mean value 3.933), while *E. coli* was the least susceptible (with MIC, 50±0.00-, MBC, 100-mg/mL, in the agar dilution; MIC, 25±0.00-, MBC, 50-mg/mL in the broth dilution and a significant mean value of 14.70). The stem-bark extracts was also significantly more active than the latex extracts *P*= .05 with significant mean values of 13.48 and 19.53 respectively. In the time-kill assay, all (100%) the organisms tested were killed by 50% methanol extract of *E. abyssinica* at concentrations equivalent to 1MIC- 4MIC.
Conclusion: *E. abyssinica* extracts showed considerable antibacterial activity against the bacterial species tested. These findings authenticate the folklore use of *Euphorbia abyssinica* for broad spectrum treatment of bacterial infections.

Keywords: Antibacterial activity; Euphorbia abyssinica extracts.

1. INTRODUCTION

Plants are a limitless gift of nature to humans and they possess very appreciative values and roles. They have stood the test of time in the life of man since creation. All over the world, they are hugely exploited for food, fuel, timber, medicine etc. [1]. The limitless value of plants is reflected in the prophylactic effect of plants eaten raw as food by herbivores. These animals have no organized clinic and yet live in the wild with little or no health problems all year round. The natural endowment of plants with numerous metabolites and bioactive compounds makes them good sources of therapeutic agents capable of replacing synthetic antibiotics; For example, Salversan and Penicillin are synthetic drugs formerly used for the treatment of Syphilis and *Staphylococcus aureus* infections, respectively, but which became less preferred because these pathogens developed resistance to the drugs [2]. According to Dr. Gordon M. Cragg of the Natural Products Research, US National Cancer Institute, "the development of clinically effective anticancer agents such as taxol, and discovery of potential anti-AIDS agents such as michellamine B demonstrate the value of plants as sources of potential new drugs." Since this first discovery of antibiotic resistance by *S. aureus*, multidrug resistance has become a major global challenge. A great number of diseases are becoming untreatable because the microorganisms have acquired drug resistance genes through lateral gene transfer. Harnessed by the extensive, massive overuse and misuse, of the antibacterial agents that constitute the principal basis for human infection therapy, multidrug resistance has led to the ineffective eradication of many serious infectious diseases [3]. However, to overcome this problem, effective and safe alternatives must be sourced to combat this emergent bacterial resistance [4-10]. Thus researchers have resolved to drift their attention towards plant sources for alternative lead compounds. This can be justified by their being environment friendly, relatively safe, cheap, easily available, and affordable for human use. Also there have been numerous reports on the susceptibility of resistant bacterial strains to antimicrobials of plant origin [11] as well as great successes recorded in folklore medicine [12].

The folklore applications of these plants remains noteworthy [13] and can be traced to have been as far back as the existence of man [14,15]. *Euphorbia abyssinica*, commonly known as *Kechieh Bih* by the Kendem people of south-West Cameroon and or as palm bob or desert candle, belongs to the genus *Euphorbia*, family, *Euphorbiaceae,* which comprises about 300 genera and 5000 -7500 species [15,16]. This genus of about 2000 known species, includes shrubs, trees and some succulent ones resembling cacti [16], has been noted to have significant anti-inflammatory, analgesic, haemostatic (stop bleeding) and wound healing

properties [17]. Significantly in folklore medicine, *Euphorbia condylocarpa* is used to cure skin diseases, gonorrhea, costiveness, and migraine [15]. Traditionally the leaves and stem of *Euphorbia helioscopia* are used as febrifuge and vermifuge, its seeds oil as purgative, seeds combined with roasted pepper, as anti-cholera and roots as anthelminthic [18]. Also, antibacterial, antifungal, antiviral, vasodepressor, phyto-toxicity antioxidant, anticancer, anti-asthmatic and molluscicidal activities have been reported [18-24]. The work reported here seeks to authenticate some of these folklore claims of treating bacterial infections with *E. abyssinica*, by investigating the phytochemical constituents and antibacterial susceptibility profile of extracts of this plant.

2. MATERIALS AND METHODS

2.1 Collection of Materials

Euphorbia abyssinica stem bark were collected from Kendem in the south West region of Cameroon and authenticated in the Department of Botany, University of Nigeria Nsukka where the voucher specimen (two) has been deposited. The plant bark was rinsed thoroughly in running tap water, cut into tiny pieces and air dried in the dark. The dried material was then ground to powder in a mortar, weighed and stored in plastic bags in the dark. The latex or sap of *E. abyssinica* was collected by cutting open, parts of the bark on the stem and branches of whole standing plant. A container was connected to the bottom of the opening from which the latex dripped into the container. It was then allowed to dry in the water bath at 56℃ and stored in a close capped bottle pending its use.

2.2 Extraction of Plant Materials and Phytochemical Analysis of Extracts

This was carried out using the method described in [1]. A 100 g weight of powdered plant material was soaked in 400 mL of solvent (absolute methanol, water or 50% methanol) in a 1-litre conical flask covered with cotton wool plugs. The flask was shaken vigorously at first and then intermittently for 24 hours leaving it in a water bath maintained at 40℃ between the intervals of shaking. The mixture was filtered, first through three layers of clean muslin cloth, and then through Whatman no 1 filter-paper. The filtrates were evaporated to dryness in a water bath at 56℃ and the percentage yield of the crude extracts determined.

The phytochemical analysis of the plant extracts was carried out according to the methods described by [25]. Alkaloids, Flavonoids Tannins, Saponins, Cardiac glycosides, Glycosides, Proteins, Carbohydrates, Steroids, Reducing sugars, Antracene glycosides, were the classes of phytochemicalconstituents investigated in the study.

2.3 Test Organisms

Four Bacterial strains (*Staphylococcus aureus, Escherichia coli, Salmonella typhi,* and *Pseudomonas aeruginosa*) all collected from the Microbiology laboratory of the University of Nigeria Teaching Hospital, Enugu, were used in the study.

2.4 Determination of Antimicrobial Activity of Plant Extracts

Each test bacterial inoculum was standardized according to the National Committee of Clinical Laboratory Standards [26] to correspond to 0.5 MacFarland Standard (approximately 0.5 x 10^6–1.0 x 10^8CFU/mL). The turbidity of the inoculum of a given test bacterial strain was adjusted for each batch of tests.

Susceptibility testing of bacteria was done using the Agar Well Diffusion method. A 1.0 mL amount of an 18 hour culture of bacteria, adjusted to 0.5 MacFarlane standards, was put into clean sterile Petri dishes and 19 mL of molten nutrient agar (at 45℃) added; then each plate was gently swirled several times to evenly spread and properly mix the bacteria cells and the agar medium. The content of the plates were allowed to stand on a flat, undisturbed surface to solidify. Wells of approximately 6 mm diameter and 2.5 mm depth were bored on the bacteria-seeded agar medium, using a sterile cork borer. A 0.5 mL volume of the 100 mg/mL reconstituted plant extract was pipetted into one of the holes. Then 0.5 mL of sterile water was put into another hole as a negative control while 0.5 mL of gentamycin 10 µg/mL was put into another hole as a positive control. The plates were left to stand for one hour for pre-diffusion of the extract to occur and then they were incubated at 37℃ for 18-24 hours. Zones of inhibitions were measured to the nearest mm. Extracts which showed appreciable inhibition zone diameter in the test were chosen for further studies [1].

Determination of Minimum Inhibitory Concentrations (MIC) was carried out

using the standard methods of European Committee for Antimicrobial Susceptibility Testing [27].

The Minimum Bactericidal Concentrations (MBC) were determined by cutting about a 2 mm diameter agar disc from the last three agar plates in each dilution showing growth inhibition and inoculating them into fresh sterile nutrient broth. The broth cultures were incubated for 18 hours at 37℃, after which 1 mL of the culture was spread over a fresh nutrient agar medium and the incubated at 37℃ for 24 hours. The least concentration in which 99.9% inhibition occurred was taken to be the MBC [18].

For the macro-broth dilution method, the Minimum Bactericidal Concentration (MBC) was determined by taking 100 µL of sample from all the tubes which did not show any growth and sub-culturing on sterile nutrient agar plates, which were then incubated at 37℃ for 24 hours. The MBC was taken as the least concentration in which 99.9% inhibition occurred.

2.5 Time-Kill Assay of *E. abyssinica* Extracts on Test Bacterial Strains

The effect of the 50% methanol extract of *E. abyssinica* was further evaluated using time-kill assay with the macro-broth dilution technique. The extracts were reconstituted in 20% Dimethyl Sulfoxide (DMSO) and diluted in nutrient broth to achieve concentrations equivalent to 0.5 MIC, 1 MIC, 2 MIC, 4 MICs values. The bacterial inoculum sizes used depended on the strain of test organism, viz; 5 x 10^8CFU/mL for *Pseudomonas aeruginosa*, 5 x10^7 CFU/mL *Enterobacteriaceae* and 1 x10^6 CFU/mL for *S. aureus* [28]. Two sets of control tubes were included for each experiment; one set was seeded with the organism in broth without extract, and the other set, blank broth with neither test organism nor extract. All the bacterial cultures were incubated under aerobic conditions at 37℃ for 24 hours. Immediately after inoculation of the tubes aliquots of 100 µL of the negative control tubes contents were taken, serially diluted in saline and seeded on nutrient agar plates to determine the count at zero hour. The same was done for the tubes which contained the test bacteria after 15 minutes, 1 hour, 3 hours, 6 hours, 9 hours, and 24 hours. After incubation, the number of emergent colonies on the inoculated agar plate was counted and the mean count (CFU) of each test organism was determined and expressed as $\log_{10}$. The Minimum Lethal Concentration (MLC) of the extract was taken as the lowest concentration that gave approximately 100% killing [1].

2.6 Statistical Analysis

The results of the antimicrobial activity of crude extract are expressed as mean ± standard deviation of the response of four replicates determinations per sample. The extract activity, the response of the microorganisms to the extracts and the control drugs were subjected to analysis of variance (ANOVA) using the Randomized Complete Block Design (Two-way analysis of variance). Duncan's New Multiple Range Test was used to separate the means that were significantly different. Statistically significant differences between groups *p <0.05 was considered statistically significant, p > 0.05 was considered as non-significant and **p <0.01 was considered highly significant.

3. RESULTS

3.1 Yields and Phytochemical Analysis of Plant Extracts

The percentage yields from 100 g of the plant materials were, 18% for stem bark absolute methanol extrat, 15% for aqueous extract, and 25% for the 50%-methanol. The yields from the latex were 13% for absolute methanol, 12% for aqueous extract and 15% for the 50%-methanol extract (Table 1).

Phytochemical Analysis of *Euphorbia abyssinica* stem bark and latex extracts showed the presence of alkaloids, flavonoids, tannins, cardiac glycosides, carbohydrates and steroids, and saponins in all the extracts (Table 2).

Table 1. Percentage yield of the plant extracts

Percentage dry weight with different solvents			Plant part
50%methanol extract	**Aqueous extract**	**Absolute methanol extract**	
25%	15%	18%	*Euphorbia abyssinica stem bark*
15%	12%	13%	*Euphorbia abyssinica* latex or sap

Table 2. Phytochemical composition the of plant extracts

E. abyssinica latex extracts			*E. abyssinica* stem bark extracts			Metabolites
50% methanol	Water	Absolute methanol	50% methanol	Water	Absolute methanol	
+	+	+	++	+	+	Alkaloids
+	+	+	+	+	+	Flavonoids
++	+	+	+++	++	+	Tannins
+	+	+	+	+	+	Saponins
++	+	+	++	+	+	Cardiac glycosides
–	–	–	–	–	–	Glycosides
–	–	–	–	–	–	Proteins
+	+	+	+	+	+	Carbohydrates
–	–	–	–	–	–	Reducing sugars
+	+	+	+++	+	++	Steroids
–	–	–	–	–	–	Antracene glycosides

+++ = *Positive, ++= Moderately positive, + = Weakly positive, - = Negative*

3.2 Evaluation of Antibacterial Activity of *Euphorbia abyssinica* Stem-Bark

The inhibition zone diameters (IZDs) of *Euphorbia abyssinica* stem-bark extract determined by the agar well diffusion method showed that all the extracts (100%) inhibited the growth of the bacteria tested but the 50%-methanol extract was significantly the most active with IZDs of 23 mm for *Staphylococcus aureus* and 19 mm for *P. aeniginosa*. The absolute methanol extracts of this plant were next to 50%-methanol in activity against all the bacterial isolates tested, with IZDs of 18 mm for *S. aureus* and *P. aeniginosa*, respectively. The aqueous extract showed the lowest activity, while *S. aureus* was the most susceptible of the bacteria tested (Table 3).

3.3 Evaluation of Antibacterial Activity of *Euphorbia abyssinica* Latex Extracts

The methanol extracts (both absolute and 50%) of *Euphorbia abyssinica* latex showed similar activity against the bacterial strains tested (IZD 18 mm for *S. aureus* and 17 mm for *S. typhi*). The aqueous extract was the least active with intermediate activity against all the bacteria strains tested (Table 3).

3.4 Minimal Inhibitory-and Minimal Bactericidal Concentrations (MIC and MBC) of the Plant Extracts

The Minimal Inhibitory and Minimal Bactericidal-Concentrations (MIC and MBC) of the extracts were comparatively determined using the agar and macro broth dilution methods, respectively. The MIC, MBC and MIC-MBC indices of the extracts of *E. abyssinica* stem-bark obtained by agar and macro broth dilution against the tested bacteria showed that the 50%-methanol extract exhibited its highest activity on *Staphylococcus aureus* both in the agar dilution (MIC, 10.93 mg/mL; MBC, 25 mg/mL and MIC-MBC index of 0.44) and in the macro broth dilution (MIC, 3.9 mg/mL; MBC, 12.5 mg/mL and MIC-MBC index

Table 3. Inhibition zone diameters IZDs (mm) of the plant extracts on bacteria

P. aeruginosa	*S. typhi*	*S. aureus*	*E. coli*	Plant extracts (100 mg)
				E. abyssynica* stem-bark
18±1.60	17±1.60	15±1.40	18±1.60	Absolute methanol
13±1.60	12±1.80	13±1.40	13±2.16	Aqueous extracts
19 ±1.16	18 ±2.30	17±2.30	23±1.60*	50% Methanol
				***E. abyssynica* latex**
15± 0.82	17±0.82	13±0.82	18±1.60	Absolute methanol
11 ±1.16	11 ±0.08	10 ±1.60	12±0.8 0	Aqueous extracts
17±1.16	17 ±1.40	15±1.60	18±1.60	50% Methanol
18±1.16	16±0.00	21 ±0.00	20±0.00	Gentamycin (Control) (10 µg/mL)

P=.05

of 0.31) methods (Table 5). In contrast *E. coli* was the least susceptible of the bacteria tested (see MIC, MBC and MIC-MBC index achieved by both assay methods in Table 4.

Likewise, in the broth dilution*, P. aeruginosa* was the least susceptible bacterium to the absolute methanol extract (MIC, 100 mg/mL and MBC 100 mg/mL) (Table 4).

The aqueous extract of *E. abyssinica* stem-bark showed least activity – with high MBC values for all the bacteria tested (100 mg/mL for *S. aureus* and *E. coli*, MIC-MBC indices 1.00, and 200 mg/mL for *S. typhi* and *P. aeruginosa*), in the agar dilution. In the broth dilution, it showed MBC of 100 mg/mL as well as MIC-MBC index of 0.44 for *S. aureus*, and MBC of 200 mg/mL, MIC-MBC indices of 0.50 for all the gram negative bacteria tested (Table 4).

The absolute methanol extract as well as the 50% methanol extract of *E. abyssinica* latex showed similar activities on *S. typhi* and *P. aeruginosa* (MIC 50 mg/mL, MBC 100 mg/mL), as well as on *S. aureus* (MIC 25 mg/mL and 50 mg/mL), respectively (Table 5).

From the agar dilution technique, *E. coli* was the most susceptible of the Gram negative bacteria to the 50% methanol extract, but in the broth dilution technique, *S. aureus* was the most susceptible of all the bacteria isolates tested, while *S. typhi* was the most susceptible of all the Gram negative bacteria tested. The aqueous extract showed high MIC and MBC values (100 mg/mL and 200 mg/mL) on all the gram negative bacteria tested. This was the least effective of all the extracts tested (Table 5).

The MICs, MBCs, MIC-MBC indices of the control antibiotic Gentamycin showed that the least susceptible of the bacteria was *P. aeruginosa* (MIC, 2.5 µg/mL, MBC 4 µg/mL) in the agar dilution method, and MIC 2 µg/mL, MBC 4 µg/mL in the broth dilution method, while *S. aureus*, was the most susceptible of the bacteria (MIC 0.5 µg/mL, MBC 1 µg/mL, MIC-MBC index 0.5) in the macro-broth dilution method (Tables 4 and 5).

3.5 Effect of 50% Methanol Extract of *E. abyssinica* Stem- Bark on Viable Cell Count of Bacteria Using Time-Kill (Inhibition) Assay

The effect of the plant extract on the bacterial cells was estimated as the reduction of viable cell count following exposure to a given concentration of extract or control drug

Table 4. The MICs, MBCs and MIC-MBC indices of *E. abyssinica* stem-bark extracts

Macro broth dilution method			Agar dilution method			Plant extract
MIC-MBC index	MBC (mg/mL)	MIC±SD (mg/mL)	MIC-MBC index	MBC (mg/mL)	MIC±SD (mg/mL)	
Absolute methanol						
0.50	25	12.5 ±00.00	0.38	50	18.70±7.20*	*S. aureus*
0.31	100	31.25±12.50	0.38	100	37.50±14.4	*E. coli*
0.50	50	25±0.00	0.50	100	50±0.00	*S. typhi*
1.00	100	100±0.00	0.25	100	25±0.00	*P. aeruginosa*
Aqueous						
0.44	100	43.75±12.50	1.00	100	100±0.00	*S. aureus*
0.50	200	100±0.00	1.00	100	100±0.00	*E. coli*
0.50	200	100±0.00	0.50	200	100±0.00	*S. typhi*
0.50	200	100±0.00	0.50	200	100±0.00	*P.aeruginosa*
50% Methanol*						
0.31	12.50	3.90±1.60	0.44	25	10.93±1.00*	*S. aureus*
0.50	50	25±0.00	0.50	100	50±0.00	*E. coli*
0.50	25	12.5±0.00	0.38	50	18.75±7.20	*S. typhi*
0.25	50	12.5±0.00	0.50	50	25±0.00	*P. aeruginosa*
Gentamycin(10 µg/mL)(Control)						
0.50	1	0.50±0.00	0.63	1	0.63±0.25	*S. aureus*
1.00	1	1.00±0.00	0.50	2	1.00±0.00	*E. coli*
0.50	2	1.00±0.00	0.50	2	1.00±0.00	*S. typhi*
0.50	4	2.00±0.00	0.62	4	2.5±1.00	*P. aeruginosa*

P=.05

Table 5. The MIC, MBC and MIC-MBC indices of *E. abyssinica* latex extract on bacteria

Macro broth dilution method			Agar dilution method			Plant extract
MIC-MBC index	MBC (mg/mL)	MIC±SD (mg/mL)	MIC-MBC index	MBC (mg/mL)	MIC±SD (mg/mL)	
						Absolute methanol
0.88	25	21.88±6.25	0.50	50	25±0.00*	*S. aureus*
1.00	100	100±0.00	1.00	100	100±0.00	*E. coli*
0.50	50	25±0.00	0.50	100	50±0.00	*S. typhi*
1.00	100	10±0.00	0.50	100	50±0.00	*P. aeruginosa*
						Aqueous
0.75	100	75±28.88	0.5	200	100±0.00	*S. aureus*
0.50	200	100±0.00	0.5	200	100±0.00	*E. coli*
0.50	200	100±0.00	0.5	200	100±0.00	*S. typhi*
0.50	200	100±0.00	0.5	200	100±0.00	*P. aeruginosa*
			50% Methanol*			
0.37	25	9.38±3.60	0.50	50	25±0.00*	*S. aureus*
0.50	50	25±0.00	1.00	50	50±0.00	*E. coli*
0.38	50	18.75±7.20	0.50	100	50±0.00	*S. typhi*
0.50	50	25±0.00	0.50	100	50±0.00	*P. aeruginosa*
					Gentamycin (10 µg/mL) (Control)	
0.50	1	0.50±0.00	0.63	1	6.25±0.25	*S. aureus*
1.00	1	1.00±0.00	0.50	2	1.00±0.00	*E. coli*
0.50	2	1.00±0.00	0.50	2	1.00±0.00	*S. typhi*
0.50	4	2.0±0.00	0.63	4	2.5±1.00	*P. aeruginosa*

P=.05

(gentamycin) over a period of time (hours). Fig. 1 shows that growth of *Staphylococcus aureus* was completely inhibited in 6 hours by 4 MIC and 2 MIC concentrations (15.64 mg/mL and 7.82 mg/mL), respectively, while 1 MIC (3.91 mg/mL) concentration, inhibited all the cells in 9 hours. However, 0.5 MIC (1.96 mg/mL) of *Euphorbia abyssinica* stem–bark extract could not inhibit *S. aureus* within 24 hours; instead there was resuscitation and increase in the viable cell count after 24 hours. The control drug (gentamycin) at 1 µg/mL also inhibited it in 1 hour of exposure (Fig. 1). One MIC concentration equivalent (3.91 mg/mL) inhibited it in 9 hours while a sub-inhibitory concentration (1,96 mg/mL) had no effect on the bacteria.

Fig. 2 shows that 4 MIC, 2 MIC, and 1 MIC (100 mg/mL, 50 mg/mL and 25 mg/mL,) concentration of 50%-Methanol extract of *E. abyssinica* stem-bark reduced E. coli viable cell count to undetectable levels in one hour, but at 0.5 MIC value (12.5 mg/mL), of the extract did not inhibit *E. coli* cells.

The 4 MIC and 2 MIC (50 mg/mL and 25 mg/mL) inhibited *S. typhi* completely in 1 hour like the control drug (gentamycin) did (Fig. 3). By the sixth hour, 1 MIC (12.5 mg/mL) concentration reduced the cell count to 0.9 $\log_{10}$ and to zero in 9 hours. On the other hand, 0.5 MIC (6.25 mg/mL) was not bactericidal to *S. typhi*. Instead the viable cell count increased from 7.69 $\log_{10}$ to 9.6 $\log_{10}$ within 24 hours (Fig. 3).

The exposure of *P. aeruginosa* to 4 MIC and 2 MIC (50 mg/mL and 25 mg/mL) inhibited it in 24 hours while the control drug (Gentamycin) killed it in 3 hours (Fig. 4). The 1 MIC and 0.5 MIC concentrations (12.5 mg/mL and 6.25 mg/mL) within 24 hours did not result in total inhibition of the cells i.e. some growth was still apparent but the growth was significantly lower in the 1 MIC (12.5 mg/mL) treated cells (Fig. 4).

4. DISCUSSION

Euphorbia species have been reported to be of great pharmacological [29] and medicinal [30] value. Various factors affect chemical composition of the plant which may ultimately modify its uses and activities. Antimicrobial compounds such as flavonoids and terpenoids are polar constituents which can be extracted using polar solvent systems like those which were used in this study. In this study, flavonoids,

together with tannins, alkaloids, saponins, glycosides, cardiac glycosides, anthracene glycosides, amino acids were found in extracts of *E. abyssinica* stem bark and latex. Flavonoids, tannins, saponins, sesquiterpenes, and alkaloids are known to be synthesized by plants in response to microbial infection and they serve as defense mechanisms against predation by many microorganisms, insects and herbivores [31,32]. The presence of these metabolites in other *Euphorbia species* has also been reported by [14,32,33].

The large Inhibition Zones Diameters (IZD) produced by the plant extracts against the test bacteria, especially those by the 50% methanol extract, is an indication of the potency of the bioactive components of the plant against all the test organisms. However, the susceptibility of the organisms to the plant extracts varied according to the strains and species of the organisms. The inhibition zone diameters (IZDs) of *E. abyssinica* stem-bark extract determined showed that 50% methanol extract was significantly the most active with IZDs of 23 mm for *S. aureus* and 19 mm for *P. aeniginosa*. The absolute methanol extracts of this plant showed 18 mm for *S. aureus* and *P. aeniginosa*, respectively. The aqueous extract showed the lowest activity on all the bacteria tested, while *S. aureus* was the most susceptible of the bacteria tested. Similar trend of susceptibility has also been reported by [34] on *Euphorbia milii* and by [35] in their works on *Euphorbia hirta* respectively. It is quite possible that some of the active chemical constituents of the plants were not soluble in methanol or water used as extraction solvents; hence the reason why some of the plant parts in this study did not exhibit sufficient antibiotic properties. The drying process may have also caused some conformational changes to occur in some of the chemical constituents found in these plant parts and some volatile constituents might have escaped from the plants.

The spectra of activity of these plant extracts on the microorganisms were not the same. In all the extracts used, the 50% methanol extracts of all the two plant parts were the most potent in activity. Their activities were statistically more significant (P= 0.05) than the rest of the extracts. The absolute methanol extracts of all the plant parts were also statistically more significant (P = 0.05) in activity than the aqueous extracts. This shows that the alcohol soluble components are more bioactive against the test organisms than the water soluble constituents. It is yet to be determined whether the ethanol extracts will show activity similar to methanol extracts since ethanol from the local gin (kai-kai)

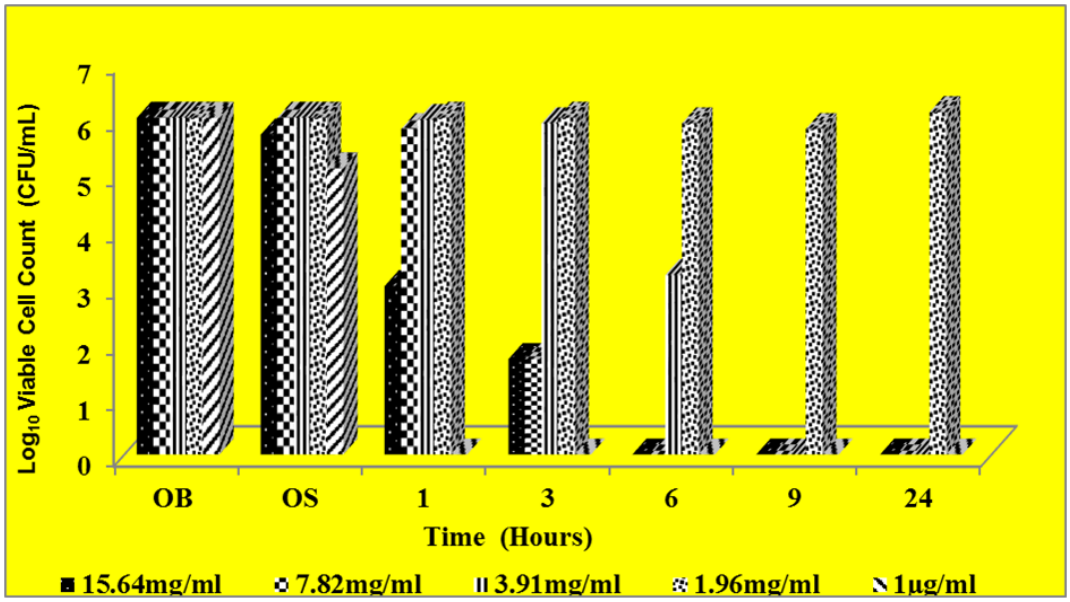

Fig. 1. Effect of 50% methanol extract of *Euphorbia abyssinica* stem- bark on viable cell count of *Staphylococcus aureus*

OB= Time of commencement of the experiment. (Zero time), OS= 15 minutes after the commencement of the experiment

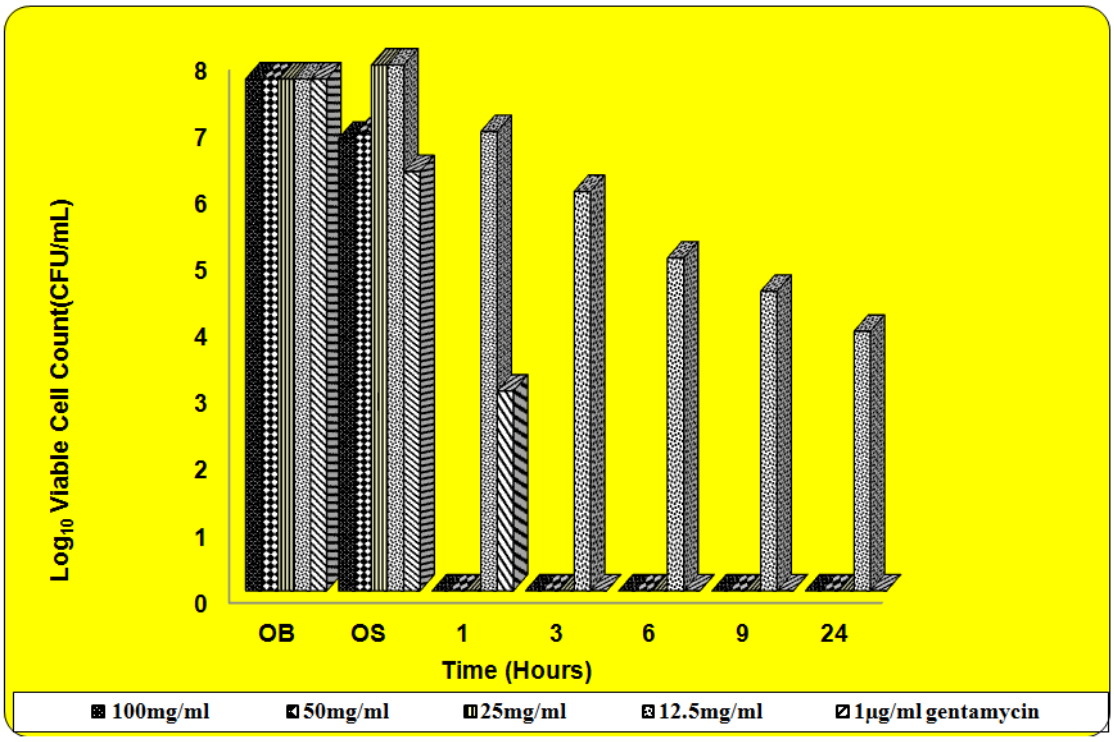

Fig. 2. Effect of 50% methanol extract of *Euphorbia abyssinica* stem- bark on viable cell count of *E. coli*

OB= Time of commencement of the experiment. (Zero time), OS= 15 minutes after the commencement of the experiment

is the type of alcohol used in folklore to prepare consumable concoctions. However, the actual constituents of kai-kai have not been published; rather they are speculated to contain mainly ethanol with traces of other alcohols, including methanol and acetone. However, it has been observed that the extraction of active constituents and, therefore, the efficacy of herbal remedies depend on the extracting solvent, geographical location, and soil characteristics among other factors [36]. A similar study reported by [33] also showed the descending sequences of antimicrobial activity of various samples and extracts of *Euphorbia hirta* and *Euphorbia thymifolia* against microorganisms studied (fresh latex, fresh juice, methanol extract, ethanol extract, DCM extract, aqueous extract and diluted latex extract (no activity). Also, [37] in their work on *E. neiirifolia* observed that water and ethyl acetate extracts exhibited very less activity against some bacterial organisms tested.

From the MIC values obtained in this study, there was no significant difference between the macro-broth dilution method and the agar dilution method. The lowest MICs and MBCs, signifying high efficacy, were demonstrated against *S. aureus* while highest MIC and MBC values, showing lower efficacy, were recorded against *S. typhi, E. coli* and *P. aeruginosa* with all the extracts tested. Interestingly bioactivity has also been reported for aqueous and methanol extracts of other *Euphorbia species* [18,32,38-42]. The crude extracts of *E. abyssinica* inhibited the growth of such agents of diarrhea diseases as *E. coli,* and *S, typhi*, as well as notoriously drug resistant pathogens such as *P. aeruginosa* and *S. aureus*. These bacteria which usually display above average resistance to most antibiotics and antibacterial agents, have virulence factors from a restrictive outer membrane barrier and trans envelope multidrug resistance efflux pumps, which are responsible for a significant level of resistance to antibiotics in pathogenic bacteria [1]. Gram-negative MICs were found to be about four fold higher than those of the other microbial strains, presumably because the plant extracts may have been effluxed by Mex EF-Oprn system as in *P. aeruginosa* and sulfa-methoxazole, thereby reducing the effective concentration of the extract.

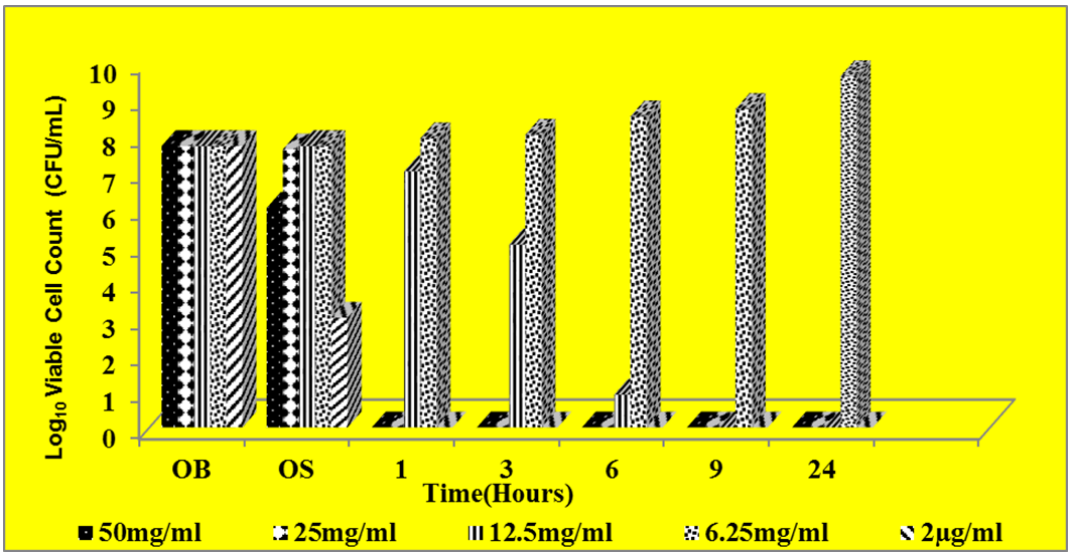

Fig. 3. Effect of 50% methanol extract of *Euphorbia abyssinica* stem- bark on viable cell count of *Salmonella typhi*

OB= Time of commencement of the experiment. (Zero time), OS= 15 minutes after the commencement of the experiment

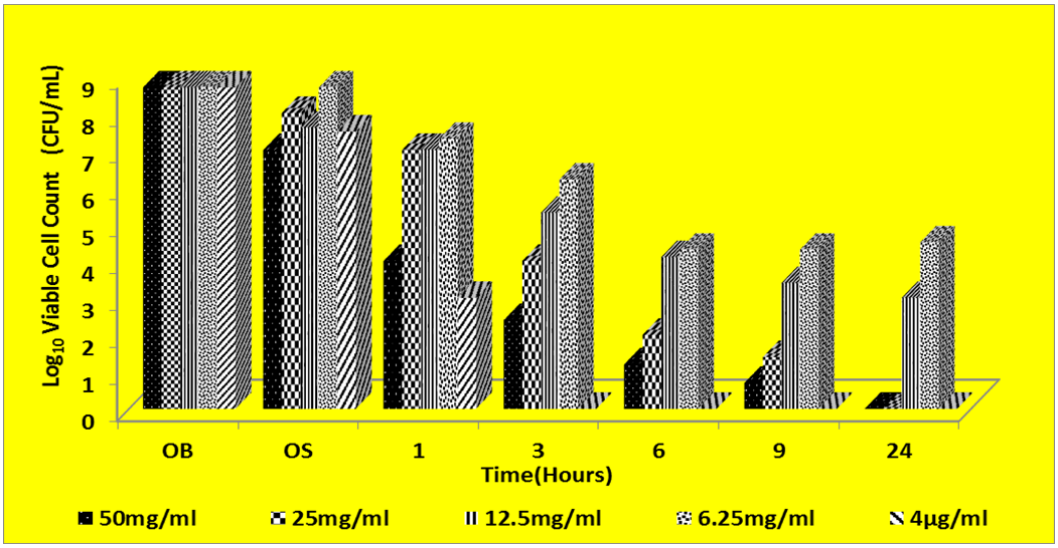

Fig. 4. Effect of 50% methanol extract of *Euphorbia abyssinica* stem- bark on viable cell count of *Pseudomonas aeruginosa*

OB= Time of commencement of the experiment. (Zero time), OS= 15 minutes after the commencement of the experimen

Generally, the crude extracts showed increasing levels of inhibition with increasing concentrations in the Time kill assay.

In terms of spectrum of activity 50% methanol extract showed some results that are comparable to the control drugs (gentamycin) used in this study. However, the control drugs were effective in microgram concentrations while the plant extracts were effective in milligram concentrations. Therefore actual comparison between the control drugs and the extracts would await isolation, purification and determination of molar concentrations of the pure active ingredients of these plants extracts [1].

5. CONCLUSION

The determination of the antimicrobial activity of *Euphorbia abyssinica* stem (bark and Latex extracts) extract included the 50% methanol, absolute methanol and aqueous extracts of these plant parts. The antimicrobial activity variously exhibited by the 50% methanol extracts of all the two plant parts tested, is significant. This is because it validates the popular traditional uses of dilute alcohol concoctions of medicinal plant preparations in ethno medicinal practice in south-West region of Cameroon. Secondly, the results indicated that these herbs used in traditional medicine have selective antimicrobial activities. Thus, the microorganisms which were susceptible to these extracts are those often associated with wound and ear infections, urinary and gastrointestinal tract infections as well as pyrexia of unknown origin. This explains the discriminate uses of these plants in the treatment of particular ailments. These findings provide evidence that *E. abyssinica* is a strong candidate for therapy against multidrug-resistant Gram-negative and Gram positive bacteria especially *S. aureus* and *P. aeruginosa*. This also validates the ethno medicinal uses of *E. abyssinica* for the purpose of exploration and exploitation of sources of potential broad spectrum antimicrobial therapeutic agents. More works therefore, need to be done with the view of its use for *in-vivo* studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tarh JE, Okafor JI, Iroegbu CU. Evaluation of extracts of *Coleus* species for antibacterial activity. Afr J Biotech. 2015; 14(2):125-132.
2. George AW, Lechtman DM. Microbiology. Fourth edition; United States of America: New York; 1984.
3. Khalil AA, Mohamad A, Hassan H, Hani O. Antibacterial activity of four herbal extracts against methicillin resistant bacteria isolates collected from Almadinah Hospitals, Saudi Arabia. IJASR. 2015; 3(1):34-40.
4. Abbas RZ, Iqbal Z, Khan MN, Zafar MA, Zia MA. Anticoccidial activity of *Curcuma longa* L. in broiler chickens. Braz Arch Biol Technol. 2010;53:63-67.
5. Singh P, Shukla R, Prakash B, Kumar A, Singh S, Mishra PK, et al. Chemical profile antifungal, antiaflatoxigenic and antioxidant activity of *Citrus maxima* Burm and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. Food Chem Toxicol. 2010;48:1734-1740.
6. Abbas RZ, Munawar SH, Manzoo Z, Iqbal Z, Khan MN, Saleem MK, et al. Anticoccidial effects of acetic acid on performance and pathogenic parameters in broiler chickens challenged with *Eimeria tenella*. Pesq. Vet. Bras. 2011; 31:99-103.
7. Oskay M. Effects of some environmental conditions on biomass and antimicrobial metabolite production by *Streptomyces* sp., KGG32. Int J Agric Biol. 2011;13:317–324.
8. Abbas RZ, Colwell DD, Gilleard J. Botanicals: An alternative approach for the control of avian coccidiosis. World Poult Sc J. 2012a;68:203-215.
9. Abbas RZ, Iqbal Z, Khan A, Sindhu ZUD, Khan JA, Khan MN, et al. Options for Integrated strategies for the control of Avian Coccidiosis. Inter J Agric Biol. 2012b;14:1014-1020.
10. Zaman MA, Iqbal Z, Abbas RZ, Khan MN. Anticoccidial activity of herbal complex in broiler chickens challenged with Eimeriatenella. Parasitol. 2012;139(2): 237-243.
11. Tajkarimi MM, Ibrahim SA, Cliver DO. Antimicrobial herb and spice compounds in food. Food Cont. 2010;21:1199-1218.
12. Dubey RK, Dubey CS, Jayaveera KN. Human vaginal pathogen inhibition studies on aqueous, methanolic and saponins extracts of stem barks of *Ziziphus mauritiana*. Inter J Pharm Sci Res. 2011; 2(3):659-663.
13. Rajeshwari S, Balakrishnan A, Thenmozhi M, Venckatesh R. Antimicrobial activity of *Aegle marmelos, Ruta graveolens, Opuntia dellini, Euphorbia royleana* and *Euphorbia antiquorum*. J Pharm Res. 2011;4(5):1507-1508.
14. Okeniyi SO, Adedoyin BJ, Garba S. Phytochemical screening, cytotoxicity, antioxidant and antimicrobial activities of stem and leave extracts of *Euphorbia heterophylla*. Bull Envi Pharm Life Sci. 2012;1(8):87–91.
15. Sepideh M, Vahid A, Seyed ASS, Erfan H, Hamidreza J, Reza AC, et al. Antimicrobial activity of methanolic root extracts of *Euphorbia condylocarpa* against

pathogenic bacteria. Adv Stud Biol. 2015; 7(2):55–64.

16. Devanaboyina NA. Mounisha BB, Sireesha G, Vijaya KK, Sri Ramavenkata RM. Antimicrobial studies on flowers of *Euphorbia milii*. Sch. Res. Lib. Der Pharm Lett. 2015;7(3):196-204.
17. Daud M, Hidayatullah S, Khan H, Faheema FU. Antibacterial activity of leaf extract of *Euphorbia cf. serrate* against known human pathogenic bacteria. Am-Eur, J. Agric. Env. Sc. 2014;14:07-10.
18. Saleem U, Saleem M, Ahmad B, Hussain K, Ahmad M, Bukhari NI, et al. *In-Vitro* antimicrobial susceptibility testing of leaves methanol extract and latex of *Euphorbia helioscopia* using agar well diffusion and broth dilution methods. JAPS. 2015;25(1): 261-267.
19. Abbas RZ, Iqbal Z, Blake D, Khan MN & Saleemi MK. Anticoccidial drug resistance in fowl coccidia: The State of Play Revisited. Wld Poult. Sc. J. 2011a;67:337-350.
20. Nikolova M, Evstatieva L, Nguyen TD. Screening of plant extracts for antioxidant properties. Bot. Serb. 2011;35(1):43-48.
21. Khan AM, Qureshi RA, Gillani SA, Ullah F. Antimicrobial activity of selected medicinal plants of Margalla Hills, Islamabad. Pak J Med Pl. Res. 2011;18:4665-4670.
22. Maoulainine LBM, Jelassi A, Hassen I, Boukhari A. Antioxidant proprieties of methanolic and ethanolic extracts of *Euphorbia helioscopia*, (L.) aerial parts. Int Food Res J. 2012;19(3):1125-1130.
23. Wang ZY, Liu PH, Zhang YC, Guo LQ, Li ZX, Shi XF. Anticancer potential of *Euphorbia helioscopia* L. extracts against human cancer cells. The Anat. Rec. 2012; 295:223-233.
24. Saleem U, Ahmad B, Hussain K, Ahmad M, Bukhari NI. Estimation of antioxidant power in various extracts of *Euphorbia helioscopia* L. with five different in vitro antioxidant models. Asian J Chem. 2014; 26(4):1241-1245.
25. Trease GE, Evans WC. Pharmacognosy. 14th. Edition. London: Philadelphia: WB Saunders: 996.
26. National Committee for clinical laboratoy standards method for dilution susceptibility testing of bacteria that grows aerobically. Approved standards M7-a2 and information supplement M100-S10 NCCLS; Wayne, Pa. USA; 1997.
27. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of MICs of antibacterial agents by agar dilution. Clin Microbiol Inf. 2000;6(9):509-515.
28. Barry AL, Lasner RA. *In vitro* methods for determining MLC of antimicrobial agents. Am J Clin Pathol. 1979;71:43-91.
29. Citoğlu GST, Özbilgin S. Uses of some *Euphorbia species* in traditional medicine in Turkey and AAtheir biological activities. Turk J Pharm Sci. 2012;9(2):241-256.
30. Kirbag S, Pınar E, Fikriye Z, Ayşe NG. Antimicrobial activities of some *Euphorbia* species. Afr J Trad Compl Alt Med. 2013;10(5):305-309.
31. Shanmugapriya P, Roziahanim M, Suthagar P, Wei CL, Surash R. Antimicrobial activity and cytotoxicity evaluation of *Euphorbia hirta* (L.) extracts from Malaysia. Apc. Proc. 2012;2:80–85.
32. Yeruva RR, Haranadha VB. Evaluation of antimicrobial and antibacterial activity of *Euphorbia nivulia*. Int. J. Pharm. Sc. Rev. Res. 2016;38(2):48:262-265.
33. Musaddique H, Umer F, Muhammad R, Hazoor B, Abdul M, Imran AK, et al. Antimicrobial activity of fresh latex, juice and extract of *Euphorbia hirta* and *Euphorbia thymifolia*– an In vitro comparative study. Int. J. Pharm. Sc. 2014;4(3):546-553.
34. Pradyutha AC, Uma MRV. Phytochemical screening and antimicrobial evaluation of *Euphorbia milii* leaf extracts. Wld J. Pharm. Res. 2015;4(8):1626-1633.
35. Adjeroh LA, Ajuruchi VC, Nnokwe JC, Azuwike CO, Mgbemena IC. Preliminary phytochemical screening and antibacteria/ antifungal activities of leaf extracts of *Euphobia hirta*. J Natl Sc Res. 2015;5(16): 153-158.
36. Ughachukwu PO, Ezenyeaku CCT, Ochiogu BC, Ezeagwuna DA, Anahalu IC. Evaluation of antibacterial activities of *Euphorbia heterophylla*. IOSR J Dent Med Sc. 2014;13(11):69-75.
37. Kumara SM, Neeraj P, Santosh D, Anuradha M. Phytochemical and antimicrobial studies of leaf extract of *Euphorbia neriifolia*. J Med Plants Res. 2011;5(24):5785-5788.
38. Manoj G, Dinakar S, Badri P, Nagori G. Analysis and antimicrobial action of latex of *Euphorbia caducifolia*. J Intercult Ethnopharmacol. 2012;1(2):119-123.

39. Singh G, Kumar P, Alka J. Antibacterial potential of sterols of some medicinal plants. Int J Pharm Pharm Sc. 2012;4(3): 159-162.
40. Lacuna MDLG, Carmona ML, Amparado BB, Daclan MAF, Ranido LA. Antimicrobial activity of supercritical fluid extracts of two philippine medicinal plants, *Psidium guajava* and *Euphorbia hirta*: Implications to community health. Adv Agric and Bot- Int. J. Biofux Soc AAB Bioflux. 2013;5(1):1-12.
41. Shahrzad NS, Mehdi R, Hamed A, Hassan G. Evaluation of antimicrobial effects of *Euphorbia cyparissias* extracts on intramacrophages *Salmonella typhi*. J. Biol. Act. Prod. Nat. 2013;3:1:64-71. DOI: 10.1080/22311866.2013.782751
42. Addai-Mensah D, Oduro-Mensah D, Konona-Ang PM. *In vitro* antibacterial activity of peg formulations of crude extracts of *Cleome viscosa*, *Tamarindus indica* and *Euphorbia hirta.* Res J Microbiol. 2016;11:202-207.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18862