RESEARCH ARTICLE

Multidrug resistant bacteria are sensitive to *Euphorbia prostrata* and six others Cameroonian medicinal plants extracts

Igor K. Voukeng¹, Veronique P. Beng² and Victor Kuete¹*

Abstract

**Background:** Multidrug resistant (MDR) bacteria are responsible for therapeutic failure and there is an urgent need for novel compounds efficient on them.

**Methods:** Eleven methanol extracts from seven Cameroonian medicinal plants were tested for their antibacterial activity using broth micro-dilution method against 36 MDR bacterial strains including *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Providencia stuartii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

**Results:** *Euphorbia prostrata* extract was found active against all the 36 tested bacteria including Gram-negative phenotypes over-expressing efflux pumps such as *P. aeruginosa* PA124, *E. aerogenes* CM64 and *E. coli* AG102. *E. prostrata* had minimal inhibitory concentrations values between 128 and 256 µg/mL on 55.55% of the studied microorganisms. Other plants extract displayed selective antibacterial activity.

**Conclusions:** Results obtained in this study highlight the antibacterial potential of the tested plants and the possible use of *E. prostrata* to combat bacterial infections including MDR phenotypes.

**Keywords:** Antimicrobial, Cameroon, *Euphorbia prostrata*, Medicinal plant, Multidrug resistance

Background

Bacterial multidrug-resistance is the ability of bacteria to grow in the presence of antibiotics at concentrations that were previously inhibitory. Treating infections caused by multidrug-resistant (MDR) bacteria is a challenge more and more difficult to solve within hospital units [1]. Although the resistance of bacteria to antibiotics is a natural adaptation phenomenon, the rapid emergence of MDR phenotypes is mainly due to misuse of antibiotics, which increases the selection pressure and favors the appearance MDR microorganisms [2]. In hospitals, patients infected by these bacteria stay for long time, which impacts on the cost of treatment. Faced with this crisis, it is important to develop new antibacterial molecules effective vis-à-vis of MDR bacteria and medicinal plants offer a suitable alternative. According to WHO, 80% of world population uses medicinal plants for their health needs; antibacterial potential against the multidrug resistant phenotypes of many of them like *Afro- monum citratum*, *Afromomum melegueta*, *Imperata cylindricum*, *Cinnamomum zeylanicum*, *Dioscorea bulbifera*, *Dorstenia psilurus* has already been demonstrated [3, 4]. In order to contribute to the discovery of active substances from medicinal plants, this study was designed to assess the antibacterial potential of different parts of *Aloe buettneri* A. Berger (Asphodelaceae), *Alchornea floribunda* Müll. Arg. (Euphorbiaceae), *Crinum purpurascens* Herb. (Amaryllidaceae), *Euphorbia prostrata* Ait. (Euphorbiaceae), *Markhamia tomentosa* K. Schum. (Bignoniaceae), *Viscum album* L. (Loranthaceae) and *Rauwolfia macrophylla* Ruiz & Pav. (Apocynaceae) against MDR Gram-positive and Gram-negative phenotypes.

*¹Correspondence: kuetevictor@yahoo.fr
¹ Department of Biochemistry, Faculty of Science, University of Dschang, Dschang, Cameroon
Full list of author information is available at the end of the article

© The Author(s) 2017. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Methods

Plant material and sample preparation
Different part of the investigated plants including leaves, stem, stem bark, bark or whole plant (Table 1) were harvested in different regions of Cameroon. The plants were then identified at Cameroon National Herbarium where the voucher specimens are available (Table 1). The dry powders (200 g) of each part of plants were soaked in methanol for 48 h; the filtrates obtained after filtration paper through Whatman No. 1 were concentrated under reduced pressure and the obtained extracts were kept at 4 °C for further biological tests.

Phytochemical screening
The presence of compounds belonging to different classes of secondary metabolites was determined according to described methods [5].

Chemicals for antimicrobial assay
The reference antibiotic (RA) used against bacteria were chloramphenicol and ciprofloxacin (Sigma-Aldrich, St. Quentin Fallavier, France) meanwhile the bacterial growth indicator was p-iodonitrotetrazolium chloride ≥ 97% (INT, Sigma-Aldrich).

Microbial strains and culture media
Microorganisms used in this study included 36 multi-drug-resistant strains of Gram-negative belonging to Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae, Klebsiella pneumoniae, Providencia stuartii, Pseudomonas aeruginosa species and Gram-positive bacteria belonging to and Staphylococcus aureus species (Table 3). Their bacterial features were previous reported [6]. Mueller–Hinton Agar (Sigma) was used to activate the microorganisms whilst Mueller Hinton broth (MHB; Sigma) was used for antibacterial assays.

INT colorimetric assay for MIC and MBC determinations
The minimum inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of different plant extracts were determined by a by the rapid INT colorimetric assay according to described methods [3]. Chloramphenicol and ciprofloxacin were the reference drugs for Gram-negative and Gram-positive respectively. Each plant extract was dissolved in a 5% DMSO solution; an aliquot of 100 μL was added to the wells of a microplate containing 100 μL of MHB; then serial dilution was performed. A bacterial suspension corresponding to 0.5 McFarland scale was prepared and diluted 100 times in the sterile MHB. Afterwards, 100 μL of the bacterial suspension was added to all wells and the plate was incubated at 37 °C for 18 h. Bacterial growth was detected by adding 40 μL of INT (0.2 mg/mL) and the appearance of pink color indicated bacterial growth; the lowest concentration of the extract where no color change was observed was recorded as MIC.

For the determination of MBCs, we used new 96-well plates containing 150 μL of MHB in which we added an aliquot of 50 μL from the wells corresponding to MIC as well as upper concentrations. Those microplates were incubated at 37 °C for 48 h and revelation was done as mentioned above and the lowest concentration indicating the absence of bacterial growth was considered as MBC. Each of the experiments was carried out in triplicate.

Results
The classes of secondary metabolites present in extracts of different parts of plants were detected and results are summarized in Table 2. Alkaloids, triterpenes, sterols, flavonoids, polyphenols and saponins were screened in all plant extracts. Other classes of compounds were selectively distributed.

The results of antibacterial tests are summarized in Table 3. It appears that the plant extract from E. pros trata was active against all tested bacterial strains (36/36) with MIC between 128 and 256 μg/mL on 55.55% (20/36) of the studied microorganisms. The methanol extracts of leaves and stems of V. album as well as other plant extracts displayed selective activities on MDR Gram-negative as well as on methicillin-resistant S. aureus (MRSA) strains. Leaves extract of R. macrophylla were active against 83.33% (30/36) of the tested bacteria while only 3/36 (8.33%) of the studied bacteria were sensitive to the extract from the leaves of A. floribunda. The extract of A. buettneri leaves had weak activity, its inhibitory effects being observed against 3/36 (8.33%) bacterial strains.

Discussion
The emergence of diseases due to MDR bacterial strains is a phenomenon of growing concern worldwide, being qualified by WHO as a “slow-moving tsunami” [7]. Medicinal plants are a promising alternative for the
Table 1 Information on studied species

| Plant species, (voucher number)/family | Traditional use | Part used traditionally | Potential active constituents | Previously screened activity |
|--------------------------------------|-----------------|-------------------------|------------------------------|------------------------------|
| Voukeng et al. BMC Res Notes (2017) 10:321 |

| Aloe buettneri A. Berger (59062/HNCC/Aphodeiacae) | Gastro-intestinal infections, chonic wounds, cutaneous infections, inflammations, gastric ulcers, chronic skin ulcer, cough, dysmenorrhea, food poisoning, difficult delivery, dysentery, general stomachaches, lumbar pain, regulation of menstrual cycle, functional infertility | Leaves | – | Anti-ulcer, anti-inflammatory, ovarian steroidogenesis effect, sub-acute toxicity, Analgesic effect, Antipyretic activity (18–20) |
| Alchornea floribunda Müll. Arg. (4595/HNCC/Euphorbiaceae) | Hepatitis, wounds, ringworm, eczema, pains in the heart, antidote to poison, urinary, respiratory and intestinal disorders, aphrodisiac | Leaves, bark, root | Cathecin, epicatechin, taxifolin, 5α-stigmastane-3,6-dione, 3β-hydroxyl-5α-stigmastane-24-ene, 5α-stigmastane-23-ene-3,6-dione | Antibacterial, anti-inflammatory, antioxidant, antiprotozoal, cytotoxicity (15, 21–23) |
| Citrus purpurascens Herb. (40058/HNCC/Amyralidaceae) | Pneumonia, ovarian problems, herna, wounds, dysentery, microbial infection, aphrodisiac, snake bite | Leaves, bulbs | Hippadine, pratorimine, β-O-glucopyranosides sitosterol | Antibacterial (25) |
| Euphorbia prostrata Ait. (2974/HNCC/Euphorbiaceae) | Infertility, menstrual pain, dysentery, typhoid fever | Leaves, whole plant | Prostratins A, B and C, rugosins A, D, E and G, quercetin 3-O-β-sambubioside, stigmastane-24-ene, 5α-stigmastane-23-ene-3,6-dione, luteolin | Antibacterial, anti-fungal, anti-inflammatory, antioxidant, antitumor (31–33) |
| Markhamia tomentosa K. Schum. (1974/SRFK/Bignoniaceae) | Edema, cancer, gout, scrotal elephantiasis, pulmonary troubles, general body pain | Leaves | β-sitosterol, β-sitosterol-3-O-glucoside, dehydroiso-α-lapachone, β-lapachone, tormentic acid, 2-acetynaphtho[2,3-b]furan-4,9-dione, pomolic acid, 2-acetyl-6-methoxynaphtho[2,3-b]furan-4,9-dione, β-lapachone, tormentic acid, oleandric acid, palustrine | Antibacterial, antifungal, bleeding haemorrhoids (12, 28, 29) |
| Viscum album L. (2974/HNCC/Loranthaceae) | Athereosclerosis, hypertension, cancer, headache, dizziness, palpitation | Leaves | Coniferin, syringin, eleutheroside E, syringaresinol-O-glucoside, ligalburmosides A–E, alangilignoside C, kalopanaxin D, β-amyrin acetate, β-amyrin, lupenol, lupenin and oleic acid, betulinic acid, stigmastanol, β-sitosterol, trans-a-betulin, trans-f-farnesene, oleandric acid, palustrine | Antihypertensive, cytotoxicity, vascular effects, antioxidant, antibiotic (34, 35, 37, 38) |
| Rauwolffia macrophylla Ruiz & Pav. (1697/SRFK/Apocynaceae) | Measles or itching rash, fever, swellings, rheumatism, hepatitis, pneumonia, abdominal pain, cough and toothache, headache, insomnia and palpitation of the heart, abscess, roundworm, tapeworm, hypertension, epilepsy, eye diseases, venereal diseases | Leaves, bark, roots | Reserpine, rescurminine, ajmaline, methyl reserpine, normacusine B, suaveoline, serpentine, peakeine, vomilenine, perakine, dihydroperakine, norajmaline, ajmalicine, ajmalicine, geissoschizol, pleocarpamine, oxyhimbine, aloxyhimbine, yohimbine | Antimycobacterial and Anti-bacterial activity, antioxidant (13, 44, 45) |
discovery of new anti-infective agents capable to fight against MDR phenotypes; hence, several phytochemicals have been tested against multi-resistant phenotypes [3, 8].

According to Simões et al. [9], a plant extract or phytochemicals can be considered as antimicrobials if the MIC obtain during in vitro tests is in the range 100–1000 μg/mL. The antibacterial activity obtained with *E. prostrata* extract is important as the extract was active on all MDR bacterial strains tested. MIC value of 256 μg/mL was recorded on strains over-expressing efflux pumps AcrAB-TolC (*E. coli* AG102 and *E. aerogenes* CM64) and MexAB-OprM (*P. aeruginosa* PA124) as well as against all MRSA strains. This remarkable activity on Gram-negative as well as Gram-positive bacteria may be due to the presence of phytochemicals that exhibit antimicrobial potential such as quercetin and kaempferol; in fact, their antibacterial properties’ vis-à-vis MRSA and multi-resistant *Propionibacterium acnes* were reported [10, 11]. Moreover, Tala et al. [12] have highlighted the in vivo anti-salmonella potential of this plant. The extracts from leaves of *R. macrophylla* and *M. tomentosa* exhibited better antibacterial activity than those obtained from their barks; this selective activity may be due the qualitative and/or quantitative difference in phytochemical contents of parts of the plants. The antibacterial activity obtained with *R. macrophylla* extracts may be due to the alkaloids present in this plant; in effect, Erasto et al. [13] have demonstrated the anti-mycobacterial extracts properties of alkaloids extracts from *R. macrophylla*. *Viscum album* extracts from various parts of plant were active on all bacterial species used in this study. This reflects the broad spectrum of activity phytochemical compounds available in these extracts like the triterpenes, flavonoids, alkaloids [14]. *Pseudomonas aeruginosa* strains (PA01 and PA124) were not susceptible to the leaves extract of *C. purpurascens* although the fact that this was active on 29 strains out of the 36 tested (including all the MRSA strains); Voukeng et al. [3] have highlighted the involvement of efflux pumps-type RND as the major phenomenon of resistance of Gram-negative bacteria herein studied vis-à-vis of some plant extracts. The susceptibility of the studied MDR bacteria vis-à-vis of *A. floribunda* extracts varied depending on the part of the plant used; these results corroborate those obtained by Siwe et al. [15]. Who got MIC value of 130 μg/mL and 2000 μg/mL with the methanol extracts of the leaves and bark respectively against *S. aureus* ATCC 25923. Okoye and Ebi [16] showed that fractions from leaves extract of *A. floribunda* contained mostly terpenoids, and possessed antibacterial activity against *P. aeruginosa*, *Salmonella keitambii* and *Bacillus subtilis*. Likewise, some flavonoids isolated from this plant such as taxifolin had MIC value of 225 μg/mL on the *S. sobrinus* [17].

| Plant name         | Used part and extraction yield | Alkaloids | Triterpenes | Sterols | Flavonoids | Polyphenols | Saponins |
|--------------------|--------------------------------|-----------|-------------|---------|------------|-------------|----------|
| *A. buettneri*     | Whole plant (8.15%)            | +         | +           | -       | -          | -           | +        |
| *A. floribunda*    | Leaves (4.36%)                 | -         | +           | +       | -          | -           | +        |
|                   | Stem-bark (2.88%)              | +         | +           | -       | +          | +           |          |
| *C. purpurascens*  | Leaves (6.85%)                 |           | +           | +       | -          | +           |          |
| *E. prostrata*     | Whole plant (9.14%)            | -         | -           | +       | +          | +           |          |
| *M. tomentosa*     | Leaves (8.39%)                 | -         | +           | +       | -          | +           | +        |
|                   | Bark (5.31%)                   | -         | +           | -       | +          | +           |          |
| *V. album*         | Leaves (4.97%)                 | +         | +           | +       | +          | -           |          |
|                   | Stem (14.85%)                  | +         | +           | +       | +          | +           |          |
| *R. macrophylla*   | Leaves (11.58%)                | +         | +           | -       | -          | -           | +        |
|                   | Bark (17.26%)                  | +         | +           | -       | -          | -           |          |
| Bacterial strains | Alchornea floribunda | Aloe buettneri | Euphorbia prosstrata | Rauwolfia macrophylla | Chloramphenicol (leaves) | Bark | Leaves Stem Bark Leaves Bark Chloramphenicol ATM | RA | Chloramphenicol ATM |
|-------------------|----------------------|---------------|---------------------|----------------------|------------------------|------|--------|--------|-----------------|
| **ATCC8739**     | -                    | -             | -                   | -                    | 256 (1)                | -    | -      | -      | -               |
| **ATCC10356**    | 1024 (−)             | -             | -                   | -                    | 256 (−)                | -    | -      | -      | -               |
| **AG1000**       | 1024 (−)             | -             | -                   | -                    | 256 (−)                | -    | -      | -      | -               |
| **AG102**        | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **MC100**        | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **W3130**        | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **P. aeruginosa**| 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **PA01**         | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **PA1**          | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **E. coli**      | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **E. cloacae**   | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **ATCC29916**   | 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **K. pneumoniae**| 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **K. pneumoniae**| 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **K. pneumoniae**| 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
| **K. pneumoniae**| 1024 (−)             | 512 (−)       | 256 (−)             | 256 (−)              | 512 (−)                | -    | -      | -      | -               |
### Table 3 continued

| Bacterial strains | Alchornea floribunda (leaves) | Aloe buettneri (leaves) | Markhamia tomentosa | Euphorbia prosstrata (whole plant) | Viscum album | Crinum purpurascens (leaves) | Rauwolfia macrophylla | RA: reference antibiotics; \( \text{MIC or MBC not detected up to 1024 } \mu\text{g/mL for plant extracts and 256 } \mu\text{g/mL for reference antibiotics} \); MBC values are in bracket |
|-------------------|-----------------------------|-------------------------|---------------------|---------------------------------|--------------|----------------------------|---------------------|-------------------------------------------------|
|                   | Leaves | Stem | Bark | Leaves | Bark | Leaves | Stem | Leaves | Bark | Leaves | Stem | Leaves | Bark | Chloramphenicol |
| BM47              | –      | 1024 (−) | – | 1024 (−) | – | 256 (−) | – | 256 (−) | – | 512 (−) | – | – | 256 (−) | |
| BM67              | 256 (−) | 512 (−) | – | 512 (−) | – | 256 (−) | – | 512 (−) | 1024 (−) | 1024 (−) | – | – | – | |
| BM94              | 512 (−) | 1024 (−) | – | – | – | 256 (−) | – | 512 (−) | – | 1024 (−) | – | 256 (−) | – | 128 (−) |
| S. aureus ATCC25923 | 128 (−) | 1024 (−) | – | 256 (−) | – | 128 (−) | – | 256 (−) | 256 (−) | 1024 (−) | – | – | – | 1 (8) |
| MRSA 3            | – | – | – | – | – | 1024 (−) | – | 1024 (−) | – | 1024 (−) | – | – | – | 32 (128) |
| MRSA 4            | 256 (1024) | 256 (−) | 1024 (−) | 128 (−) | – | 512 (−) | – | 256 (−) | 512 (−) | 256 (−) | 128 (−) | – | 64 (128) |
| MRSA 6            | 1024 (−) | – | 1024 (−) | 512 (−) | – | 1024 (−) | – | 256 (−) | 512 (−) | 128 (−) | 512 (−) | – | 64 (128) |
| MRSA 8            | 128 (1024) | 1024 (−) | – | 256 (−) | – | 256 (−) | – | 256 (−) | 128 (1024) | 1024 (−) | – | 128 (−) | – | 16 (64) |
| MRSA 11           | – | 256 (−) | – | 512 (−) | – | 512 (−) | – | 512 (−) | 512 (−) | 1024 (−) | 512 (−) | – | 128 (256) |
| MRSA 12           | 1024 (−) | – | – | 512 (−) | – | 128 (−) | 128 (−) | 512 (−) | 1024 (−) | – | 128 (−) | – | 32 (32) |
Conclusion
This study highlights the efficacy of some Cameroonian medicinal plants against MDR phenotypes and the results obtained can serve as preliminary test for further experiments to isolate phytochemical constituents with wide range antibacterial activity.

Abbreviations
ATCC: American type culture collection; DMSO: dimethyl sulfoxide; E. prostrata: Euphorbia prostrata; HNC: National Herbarium of Cameroon; INT: p-iodonitro-tetrazolium chloride ≥ 97% (INT, Sigma-Aldrich); MBC: minimal bactericidal concentration; MDR: multidrug resistant; MHB: Mueller–Hinton Broth; MI: minimal inhibitory concentration; RA: reference antibiotic.

Authors‘ contributions
IKV carried out the study; IKV and VK designed the experiments and wrote the manuscript; VK and VPB supervised the work; IKV and VK provided culture media, bacterial strains and other facilities. All authors read and approved the final manuscript.

Acknowledgements
Authors are grateful to the Cameroon National Herbarium (Yaounde) for plants identification, Mr. Nganou Blaise and Tala Donald Sedric for their technical support.

Competing interests
The authors declare that they have no competing interests.

Availability of data and materials
The datasets supporting the conclusions of this article are presented in this main paper. Plant materials used in this study have been identified at the Cameroon National Herbarium where voucher specimens are deposited.

Consent for publication
Not applicable.

Ethic approval and consent to participate
Not applicable.

Funding
No funding.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Received: 17 October 2016 Accepted: 21 July 2017 Published online: 25 July 2017

References
1. Cornejo-Juárez P, Vilar-Compte D, Pérez-Jiménez C, Nambendys-Silva SA, Sandoval-Hernández S, Volkow-Fernández P. The impact of hospital-acquired infections with multidrug-resistant bacteria in an oncology intensive care unit. Int J Infect Dis. 2015;31:31–4.
2. Aly NY, Al-Mousa HH, Al Asar el SM. Nosocomial infections in a medical–surgical intensive care unit. Med Princ Pract. 2008;17:373–7.
3. Voukeng KI, Kuete V, Dzoyem JP, Fankam AG, Noumedem KJA, Kuate JR, Pages JM. Antibacterial and antibiotic-potentiating activities of the methanol extract of some Cameroonian spices against Gram-negative multi-drug resistant phenotypes. BMC Res Notes. 2012;5:299.
4. Kuete V, Tepono BN, Mbaveng AT, Taayoujou LA, Meyer JM, Barboni L, Lall N. Antibacterial activities of the extracts, fractions and compounds from Dioscorea bulbifera L. BMC Complement Altern Med. 2011;12:228.
5. Harborne JB. Phytochemical methods. New York: Chapman and Hall; 1973.
6. Voukeng KI, Beng PV, Kuete V. Antibacterial activity of six medicinal Cameroonian plants against Gram-positive and Gram-negative multidrug resistant phenotypes. BMC Complement Altern Med. 2016;16:388.
7. Rein J. UN declaration on antimicrobial resistance lacks targets. Lancet. 2016;388(10052):1365.
8. Kuete V. Medicinal plant research in Africa: pharmacology and chemistry. Oxford: Elsevier; 2013.
9. Simões M, Bennett RN, Rosa EA. Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. Nat Prod Rep. 2009;26(6):746–76.
10. Lim YH, Kim IH, Seo JJ. In vitro activity of kaempferol isolated from the Impatiens balsamina alone and in combination with erythromycin or clindamycin against Propionibacterium acnes. J Microbiol. 2007;45(5):473–7.
11. Hirai I, Okuno M, Katsuma R, Ainta N, Tachibana M, Yamamoto Y. Characterisation of anti-Staphylococcus aureus activity of quercetin. Int J Food Sci Technol. 2010;45(6):1250–4.
12. Tala DS, Gatsing D, Fodouop CP, Fokunang C, Kengni F, Namekong DM. In vivo anti-salmonella activity of aqueous extract of Euphorbia prostrata Alton (Euphorbiaceae) and its toxicological evaluation. Asian Pac J Trop Biomed. 2015;5(4):310–8.
13. Erasto P, Mbawambo HZ, Nondo OSR, Lall N, Lubshagne A. Antimycobacterial, antioxidant activity and toxicity of extracts from the roots of Rauvolfia vomitoria and R. cappsi. Spatula DD. 2011(12):73–80.
14. Singh BN, Saha C, Galun D, Upreti DK, Bayry J, Kaveri SV. European Viscum album: a potent phytotherapeutic agent with multifarious phytochemicals, pharmacological properties and clinical evidence. RSC Adv. 2016;6:23837–5.
15. Siwe NK, Krause RWM, van Vuuren SF, Tantoh ND, Olivier DK. Antibacterial activity of the roots, stems and leaves of Alchornea floribunda. J Ethnopharmacol. 2014;151:1023–7.
16. Okoye FBC. Preliminary anti-microbial and phytochemical investigation of the extracts and column fractions of Alchornea floribunda leaves. J Pharm Allied Sci. 2007;4:1.
17. Kuspradini H, Mitsunaga T, Ohashi H. Antimicrobial activity of the roots, stems and leaves of Alchornea floribunda. J Ethnopharmacol. 2014;151:1023–7.
18. Telefo PB, Moundipa PF, Tchoanguep FM. Inductive effect of the leaf mixture extract of Aloe buettneri, Justicia insularis, Diclerita verticillata and Hibiscus macranthus on in vitro production of estradiol. J Ethnopharmacol. 2015;49:225–30.
19. Tan PV, Enow-Orock EG, Dimo T, Nyasse B, Kimbu FS. Evaluation of the antibacterial activity of Aloe buettneri mixture extract of Alchornea floribunda on in vitro production of estradiol. J Ethnopharmacol. 2009;45:347–7.
20. Ajaghaku DL, Obasi O, Umeokoli BO, Ogbuatu P, Nworu CS, Ilodigwe EE. Anti-ulcer and anti-inflammatory effects of hydroalcohol extract of Aloe buettneri. J Ethnopharmacol. 2016;183:105–12.
21. Ajaghaku DL, Obasi O, Umeokoli BO, Ogbuatu P, Nworu CS, Ilodigwe EE. Anti-ulcer and toxicity profile of Aloe buettneri mixture extract of Alchornea floribunda. Prod Rep. 2009;26(6):746–76.
22. Metéowogho K, Agbonon A, Eku-Gadegbeku K, Aklikokou AK, Gbessar M. Anti-ulcer and anti-inflammatory effects of hydroalcohol extract of Aloe buettneri A. Berger (Liliaceae). Trop J Pharm Res. 2008;7(1):907–12.
23. Okoye KI, Okeke OS, Okeke CI, Okoye D. Evaluation of the antibacterial activity of Aloe buettneri mixture extract of Alchornea floribunda leaves. J Ethnopharmacol. 2010;126(2):172–7.
24. Fomogne-Fodjo MCY, Van Vuuren S, Ndinteh DT, Krause RWM, Olivier DK. Antibacterial activities of plants from Central Africa used traditionally by the Bakola pygmies for treating respiratory and tuberculosis-related symptoms. J Ethnopharmacol. 2014;155(1):123–31.

25. Nkanwen ERS, Gatsing D, Ngamga D, Fodouop SPC, Tane P V. Afr Health Sci. 2009;9(4):264–9.

26. Yoshida T, Nabima O, Chen L, Liu Y, Okuda T. Ellagitannin monomers and oligomers from *Euphorbia prostrata* Art. and oligomers from *Loropetalum chinense* OLIV. Chem Pharm Bull. 1990;38(12):3296–302.

27. Mosango DM. *Euphorbia prostrata* aiton. 2008. https://www.prota4u.org/protav8.asp?h=M1,M10,M11,M12,M14,M15,M16,M18,M19,M22,M23,M25,M26,M27,M34,M36,M4,M6,M7,M98&=Euphorbia+prostrata&=Protologue. Accessed 18 Sep 2016.

28. Ali S, El-Ahmady S, Ayoub N, Singab NA. Phytochemicals of *Markhamia* Species (Bignoniaceae) and their therapeutic value: a review. Eur J Med Plants. 2015;6(3):124–42.

29. Delormar D, Calis I, Ergun F. Studies on the vascular effects of the fractions and phenolic compounds isolated from *Viscum album* ssp. album. J Ethnopharmacol. 2000;72:323–9.

30. Mollev NL. Rauvolfia caffra Sond. 2007. http://www.prota4u.org/protav8.asp?p=Rauvolfia+caffra. Accessed 22 Sep 2016.

31. Milugo KT, Omosa KL, Ochanda OJ, Owuor OB, Wamunyoyi AF, Oyugi OJ, Ochieng WJ. Antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree (*Rauvolfia caffra* Sond.): further evidence to support biotechnology in traditional medicinal plants. BMC Complement Altern Med. 2013;13:285.

32. Njau EFA, Alcorn J, Ndakidemi P, Chirino-Trejo M, Buza J. Antimicrobial and antioxidant activity of crude extracts of *Rauvolfia caffra var. caffra* (Apocynaceae) from Tanzania. Int J Biol. 2014;6(4):156–67.