Original Article

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Antimicrobial action mechanism of flavonoids from *Dorstenia* species

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ABSTRACT: Naturally occurring flavonoids have been reported to possess antimicrobial activity against a wide range of pathogens. However, the antimicrobial action mechanism of these compounds has not yet been elucidated. This study investigated the mechanism underlying the antibacterial activity of four flavonoids: 6,8-diprenyleriodictyol (1), isobavachalcone (2), 6-prenylapigenin (3) and 4-hydroxylonchocarpin (4). In addition, the toxicity of these compounds was evaluated. Determination of the minimum inhibitory concentrations (MICs) was performed by microbroth dilution method. Radiolabeled thymidine, uridine, and methionine were used to evaluate the effect of the compounds on the biosynthesis of DNA, RNA, and proteins while the sensitive cyanine dye DiS-C3-(5) (3,3'-dipropylthiadicarbocyanine iodide) was used for the effect on membrane potential. Bactericidal/bacteriolysis activities were performed by time-kill kinetic method. In the toxicity study, the numbers of survivors was recorded after injection of compounds into the hemolymph of silkworm larvae. Compounds showed significant antibacterial activity against *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA) strains with MICs values ranged between 0.5-128 µg/mL. Depolarization of membrane and inhibition of DNA, RNA, and proteins synthesis were observed in *S. aureus* when treated with those flavonoids. At 5-fold minimum inhibitory concentration, compounds reduced rapidly the bacterial cell density and caused lysis of *S. aureus*. Compounds 1, 2, and 4 did not show obvious toxic effects in silkworm larvae up to 625 µg/g of body weight. Flavonoids from *Dorstenia* species, 6,8-diprenyleriodictyol, isobavachalcone, and 4-hydroxylonchocarpin are bactericidal compounds. They cause damage of cell membrane, leading to the inhibition of macromolecular synthesis. Taking into account the *in vivo* safety and their significant antimicrobial potency, these flavonoids are promising leads for further drug development.

Keywords: Antimicrobial, flavonoids, membrane potential, macromolecules synthesis, bactericidal/bacteriolysis

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1. Introduction

Staphylococcus aureus is still an important pathogen both in community acquired and hospital associated infections (*1*). Clinical isolates of methicillin-resistant *S. aureus* (MRSA) have become the most common cause of infections among the global pathogenic bacteria and many life-threatening diseases (*2*). This situation has created new challenges in the area of drug discovery and much effort has been undertaken in the area of medicinal plants since plant sources is highly relevant for the identification of lead compounds which can result in the development of novel and safe therapeutic agents. The potential use of higher plants as a source of new drugs is still poorly explored and scientists need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts (*3*). Natural products provide majority of new drug leads for a variety of human diseases, most of the leads from natural products that are currently in development have come from either plant or microbial sources. Earlier publications have pointed out that relatively little of the world's plant biodiversity has been extensively screened for bioactivity and that more extensive collections of plants could provide many novel chemicals for use in drug discovery assays (*4*). Many plants of the genus *Dorstenia* are used in African and South American folk medicine in the treatment of illnesses such as, infectious diseases, snakebite, and rheumaticarthritis (*5*). Extensive phytochemical studies

have been carried out on a large number of *Dorstenia* species, and a variety of compounds of which flavonoids have been found widespread and some were found to exhibit interesting pharmacological activity (*6-9*). It is well known that many plant secondary metabolites such as phenolic acids, flavonoids, quinones, and alkaloids possess remarkable biological activities. Flavonoids are found as ubiquitous secondary metabolites in the plant kingdom. They have been reported to possess many useful properties, including anti-inflammatory activity, oestrogenic activity, enzyme inhibition, antiallergic activity, antioxidant activity, vascular activity and cytotoxic antitumour activity, antifungal and antibacterial activities (*10,11*). The occurrence of chalcones and prenylated flavonoids in *Dorstenia* species and their antimicrobial activity is well documented (*12-14*). Despite the great number of papers devoted to the microbial growth inhibiting effect effects of flavonoids, only a very limited number of studies are pertaining to the understanding of how these compounds exerts their antimicrobial activities. In this study, the activity of four flavonoids, 6,8-diprenyleriodictyol (**1**), isobavachalcone (**2**), 6-prenylapigenin (**3**), and 4-hydroxylonchocarpin (**4**) from *Dorsenia* species were evaluated against clinical isolates of *S. aureus*. The most active compounds (**1**, **2**, and **4**) were further investigated to determine their effect on the membrane potential dissipation and on the biosynthesis of macromolecules (DNA, RNA, and proteins) in *S. aureus*. In addition, the *in vivo* experiments were performed to evaluate the possible toxic effect of these compounds using silkworm, *Bombyx mori*.

2. Materials and Methods

2.1. *Plants and natural compounds*

The natural compounds used in this study were obtained from the chemical bank of the Laboratory of Organic Chemistry, University of Yaoundé I, Cameroon. They were isolated from plants of the genus *Dorstenia*. 6,8-diprenyleriodictyol (**1**) was isolated from the aerial parts of *D. mannii*, isobavachalcone (**2**) and 4-hydroxylonchocarpin (**4**) were isolated from the twigs of *D. barteri* while 6-prenylapigenin (**3**) was isolated from the twigs of *D. dinklagei*. The isolation procedure and the structure elucidation of compounds were performed as previously described (*6-9,13,15,16*). Chemical structures of compounds are shown in Figure 1.

2.2. *Chemicals and antibiotics*

Radiolabelled [methyl-^{3}H]thymidine and [^{3}H]uridine were purchased from Moravek Biochemical (Brea, CA, USA), and [^{35}S]methionine was purchased from the Institute of Isotopes (Budapest, Hungary). DiS-C_3-(5) (3,3'-dipro pylthiadicarbocyanine iodide) dye was purchased from AnaSpec, Inc., Otoole Avenue, USA. Amphotericin

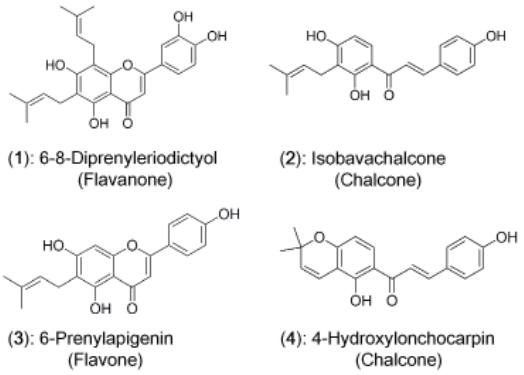

Figure 1. Chemical structure of compounds.

B, gentamicin, vancomycin, and ampicilllin (Wako Pure Chemical Industries, Osaka, Japan); rifampicin and chloramphenicol (Nacalai Tesque, Kyoto, Japan), norfloxacin and nisin (Sigma Aldrich Chemie Gmbh Steinheim, Germany) were used as reference antibiotics.

2.3. *Antimicrobial assays*

2.3.1. *Microorganisms and culture conditions*

Clinical isolates of methicillin sensitive and methicillin-resistant *S. aureus* strains (MSSA1, MRSA3, MRSA4, MRSA6, MRSA8, MRSA9, MRSA11, and MRSA12) and fungi (*Candida albicans* ATCC10231, *Candida tropicalis* pK233, *Cryptococcus neoformans* H99, and *Cryptococcus neoformans* KN99a) were used. They were obtained from the culture collection of the Laboratory of Microbiology, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Japan. Luria Bertani 10 (LB10) agar medium or Muller-Hinton agar (MHA) and Sabouraud dextrose agar (SDA) were used for the activation of bacteria and fungi strains respectively. They were subcultured in appropriated agar plates 24 h prior to any antimicrobial test. The Mueller Hinton broth, Cation-Adjusted with calcium and magnesium ions (CAMHB) and RPMI-1640 were used for the minimum inhibitory concentration (MIC) determination.

2.3.2. *MIC determination*

MIC was determined by broth microdilution method following the guidelines of Clinical and Laboratory Standards Institute (*17,18*).

2.4. *Antimicrobial action mechanisms study with S. aureus*

2.4.1. *Membrane permeabilization assay*

The effect of the compounds on the membrane potential was tested using membrane potential-sensitive dye diS-C3-(5) (*19*). Briefly, *S. aureus* MSSA1 was allowed to grow in LB10 at 37°C to an OD_{600} of 0.5-0.6. The

cell suspension was centrifuged at 10,000 rpm for 10 min and at 4°C, then washed twice in buffer (5 mM HEPES, pH 7.2; 5 mM glucose) and the OD_{600} adjusted to 0.05 with the same buffer. A 2 mL of this suspension was placed in a 1-cm cuvette; stock solution of diS-C3-(5) and compounds were added to give a final concentration of 0.4 µM and 5-fold MIC respectively. Changes in fluorescence were monitored with FP-6200 spectrofluorometer at an excitation wavelength of 622 nm and an emission wavelength of 670 nm.

2.4.2. *Effect on DNA, RNA, and proteins synthesis in S. aureus*

Metabolic incorporation of (methyl-^{3}H)thymidine, (^{3}H) uridine, and (^{35}S)methionine into cellular DNA, RNA, and protein respectively, was used to evaluated the effect of compounds on macromolecules synthesis as previously described by Ferrari and Widholm (1973) (*20*) and modified by Paudel *et al*. (2012) (*21*).

2.4.3. *Bactericidal and bacteriolysis activities*

Bactericidal and bacteriolysis activities were determined by time-kill kinetic method as described by Ooi *et al*. (2009) (*22*), with slight modifications. For bacteriolysis experiment, full growth of *S. aureus* MSSA1 in MHB were diluted 100 times and incubated at 37°C to produce an OD_{600} of 0.8 as starting inoculum. Compounds were added to give a final concentration of 5 × MIC and incubated at 37°C with shaking, then 100 µL were removed from each tube at 0, 15, 30, 60, and 120 min and the optical density measured at 600 nm. For bactericidal activity, the above mentioned bacteria suspension was diluted 100 times and incubated at 37°C to produce an OD_{600} of 0.3, then at different time intervals, 100 µL were removed from each tube; serially tenfold diluted and plated onto MHA plates, to determine the viable number of colony-forming units (cfu) per mL. Samples of 10^2 cfu/mL were below the limit of detection. Vancomycin, gentamicin and nisin were used as positive controls and no-drug tubes were also included.

2.5. *Toxicity study in silkworms*

The toxicity of compounds against silkworms was evaluated as described by Hamamoto *et al*. (2009) (*23*). Briefly, fifth instar silkworm larvae (*Bombyx mori*) were fed on the 1st day and reared overnight at 27°C. The test compounds were dissolved in saline containing 20% DMSO, then 50 µL of various concentrations were injected into the hemolymph (n = 10 per group), affording doses of 125, 250, 375, and 625 µg/g of larvae. After 24 h, the survival rates of silkworms were recorded.

3. Results

3.1. *Antimicrobial activity of flavonoids derived from Dorsenia plants*

Results of the antimicrobial assay are depicted in Table 1. All the compounds showed prominent antimicrobial activities against the tested fungal and bacterial pathogens. Compounds **1**, **2**, and **4** showed significant antibacterial activity against *S. aureus* including MRSA strains, with MICs values ranged between 0.5-16 µg/mL. *S. aureus* MSSA1 exhibited comparable sensitivity towards compound **1** and two reference antibiotics gentamicin and norfloxacin (MIC of 0.5 µg/mL). Remarkable potent antifungal activity was observed against *C. neoformans* H99 strains; MIC of compound **4** against this strain was similar to that of amphotericin B (MIC of 0.5 µg/mL). Taking into account the medical importance of the tested microorganisms, this result can be considered as promising in the perspective of new antibiotic drugs development. For natural compounds that significantly inhibit the growth of microbial pathogens to be an attractive alternative for conventional antimicrobials for

Table 1. Minimal inhibitory concentration (MIC) of compound 1, 2, 3, 4, and reference antibiotics

Microorganisms	MIC (µg/mL)											
	(**1**)	(**2**)	(**3**)	(**4**)	Van	Gen	Nis	Rif	Amp	Chl	Nor	AmB
S. aureus MSSA1	0.5	2	16	4	0.25	0.5	128	0.015	4	8	0.5	nd
S. aureus MSSA3	4	16	32	1	2	2	–	0.06	32	16	1	nd
S. aureus MRSA4	1	4	32	8	2	64	–	0.03	64	64	0.5	nd
S. aureus MRSA6	4	8	64	4	2	–	–	0.12	32	4	2	nd
S. aureus MRSA8	1	4	32	4	2	–	128	0.06	–	16	1	nd
S. aureus MRSA9	4	4	32	4	2	–	128	0.06	64	8	4	nd
S. aureus MRSA11	4	4	32	4	2	1	–	0.12	64	16	2	nd
S. aureus MRSA12	4	4	16	4	1	–	128	0.06	128	8	1	nd
C. albicans ATCC10231	128	–	64	–	nd	nd	nd	nd	nd	nd	nd	0.25
C. tropicalis pK233	–	–	–	–	nd	nd	nd	nd	nd	nd	nd	0.5
C. neoformans H99	1	2	–	0.5	nd	nd	nd	nd	nd	nd	nd	0.5
C. neoformans KN99a	–	–	–	–	nd	nd	nd	nd	nd	nd	nd	0.5

Chl, chloramphenicol; Amp, ampicillin; Van, vancomycin; Nor, norfloxacin; Gen, gentamicin; Nis, nisin; Rif, rifampicin; AmB, amphotericin B; nd, not determined; –, MIC > 128 µg/mL.

their application in therapy, it is important to establish the mechanism of their antimicrobial activity.

3.2. *Membrane permeabilization assay*

Membrane depolarization was determined using potential-sensitive dye diS-C3-(5). As shown in Figure 2, the addition of compounds **1**, **2**, and **4** induced an increase of fluorescence. Cell hyperpolarization results in uptake of the diS-C3-(5) dye molecules by the cells, thus decrease the fluorescence, while depolarization results in the release of dye thus increase the fluorescence. Membrane depolarization occurred was lower compared to nisin used as reference antibiotics.

3.3. *Effect on DNA, RNA, and proteins synthesis in S. aureus*

The metabolic incorporation of isotope-labeled uridine, thymidine, and methionine into the corresponding macromolecules in *S. aureus*, were examined to measure the biosynthesis of nucleic acids and protein. As shown in Figure 3-5, compounds **1**, **2**, and **4** induced substantial inhibiting effect on macromolecules synthesis. A slight amount of protein synthesis was observed in the presence of compound **4**. Compounds inhibited DNA and RNA synthesis to an extent similar to that of the reference antibiotics norfloxacin and rifampicin, respectively. Thus, we speculated that, the membrane damaging induced by compounds lead to the bacterial death.

3.4. *Bactericidal and bacteriolysis activities*

The result of time-kill assay is presented in Figure 6. A perusal of this figure shows a rapid decrease of survival number of *S. aureus* MSSA1 strain in the presence of compounds **1**, **2**, and **4**. Survived number cfu/mL of bacterial cells dropped below the detection limit (100 cfu/mL) after 15 min treatment for compounds **1** and **2**. Compared to initial inoculum, compound **4** showed 2-log reduction in cfu counts after 15 min. In contrast, killing activity by vancomycin and gentamicin was lower, giving less than 1-log reduction from the control

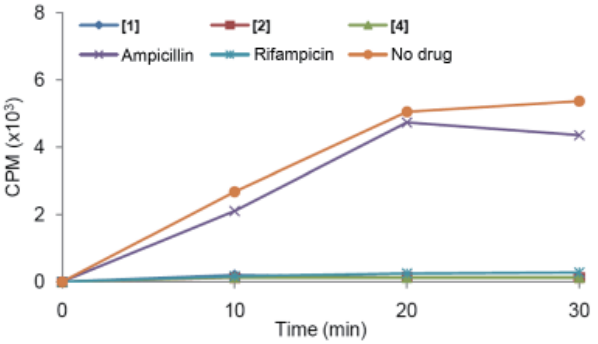

Figure 4. Effect of compounds 1, 2, and 4 on RNA synthesis.

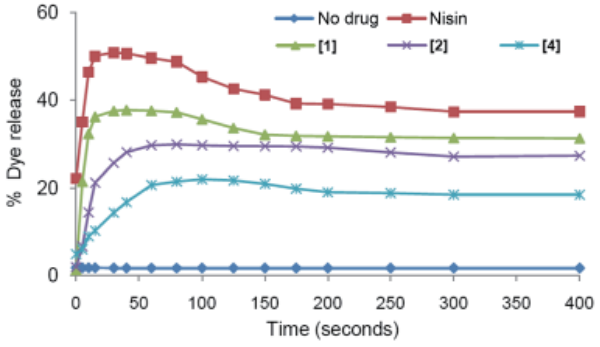

Figure 2. Effect of compounds 1, 2, and 4 on membrane permeabilization.

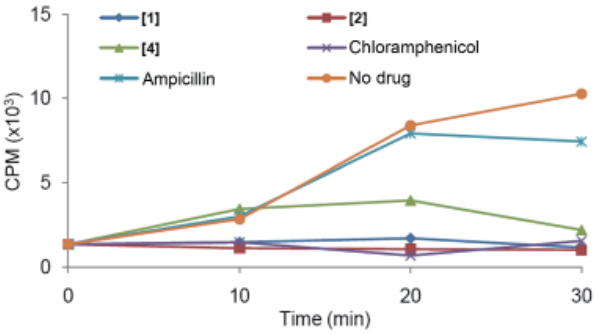

Figure 5. Effect of compounds 1, 2, and 4 on proteins synthesis.

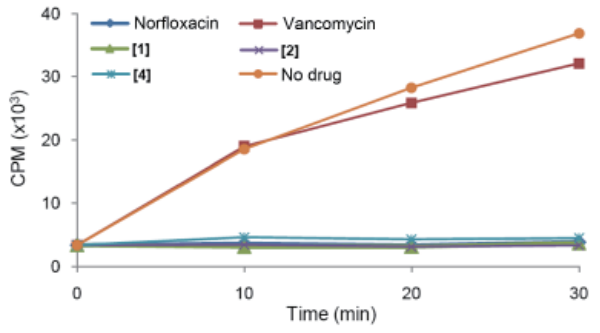

Figure 3. Effect of compounds 1, 2, and 4 on DNA synthesis.

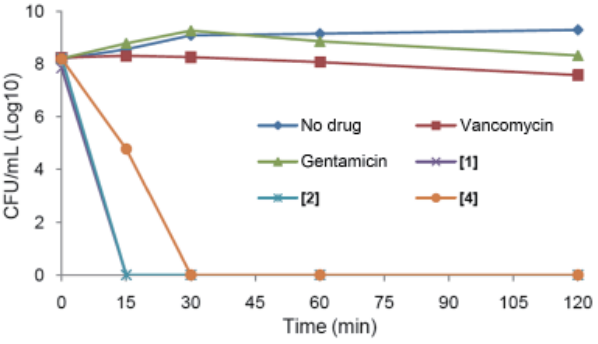

Figure 6. Time-kill curve of compounds 1, 2, and 4 against *S. aureus* MSSA1.

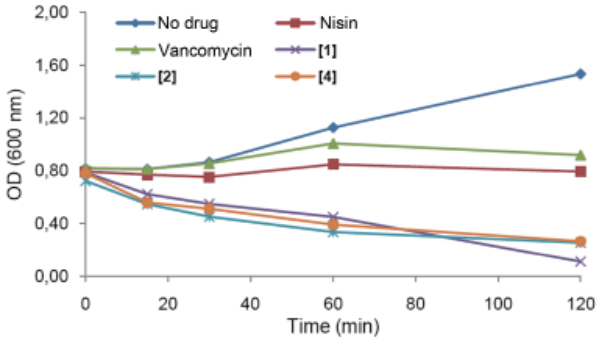

Figure 7. Bacteriolysis activity of compounds 1, 2, and 4 against *S. aureus*.

even at 120 min. The result of bactericidal activity was consistent with that of bacteriolysis activity (Figure 7) which showed a decrease in the optical density of *S. aureus* suspension treated with compounds **1**, **2**, and **4**. After 120 min, compounds **1**, **2**, and **4** induced a decline in cell turbidity of 86%, 64%, and 63%, respectively in bacteria suspension compared to the 0 time value, indicating the lysis of bacteria cells.

3.5. *Toxicity study of compounds in silkworm larvae*

The use of silkworms (*Bombyx mori*) as animal model to evaluate the toxicity of drug candidates has been proposed. Evidence is now accumulating that metabolism pathway in this invertebrate is common to that of mammals (*23*). We evaluate toxicity of compounds **1**, **2**, and **4** by using this model, no death or any obvious toxic effect was observed 24 h after injection of up to 625 µg/g of larvae, of compounds into silkworm. Based on this result, we concluded for LD_{50} values of these compounds were larger than 625 µg/g of larvae.

4. Discussion

The present study showed significant antibacterial activity against *S. aureus* including MRSA strains, this result confirm the antimicrobial potency of four flavonoids from *Dorstenia* species. Although flavonoids have been reported to possess interesting activity against a wide range of microorganisms, no study has been reported on the activity of 6,8-diprenyleriodictyol, isobavachalcone, 6-prenylapigenin, and 4-hydroxyl-onchocarpin against MRSA strains. Although no definite structure-activity relationship could be determined, some structural features that might have influenced the antimicrobial activity can be drawn from the comparison of the chemical structures of compounds with different activities. 6-8-Diprenyleriodictyol (**1**) was the most active, followed by isobavachalcone (**2**), and 4-hydroxylonchocarpin (**4**). 6-Prenylapigenin showed the lowest activity among these four flavonoids. It appears that, in general flavones (compounds **1** and **3**), 2'-hydroxyl group and the isoprenoid moiety play a greater role in increasing the antibacterial activity. 6-8-diprenyleriodictyol was 16× and 32× more active than 6-prenylapigenin against *S. aureus* MSSA1, MRSA4, and MRSA8, respectively. Published literature indicates that, the addition of an isoprenoid moiety renders higher activities in the flavonoid molecule than in the parent compounds from the pharmacological point of view (*24*). One of the proposed reasons for the enhanced biological activities of prenylated flavonoids is that the prenylation of the flavonoid core increases the lipophilicity and the membrane permeability of the compound (*25*). But this assumption was not applicable to chalcones. Athough isobavachalcone carries an isoprenoid moiety, its activity as well as that of 4-hydroxylonchocarpin were highly selective from one microbial strain to another. Nonetheless, previous studies reported that, the antimicrobial inhibitory effect of chalcones was correlated to the substitution patterns of the aromatics rings (*26*).

In membrane depolarization experiment, the membrane potential-sensitive fluorescent probe diS-C3-(5), distributes between the cells and the medium depending on the cytoplasmic membrane potential; hyperpolarization of the cell is accompanied by a decrease in fluorescence, and depolarization produced an increase in fluorescence (*27*). Our work reported here confirms the observations reported by others on the membrane damage effect of flavonoids compounds (*28,29*). When considering the overall results of bacteria viability, lysis, and membrane depolarisation, it seems that, the killing cell process induced by our compounds may be firstly due to the membrane damage, leading to the death of cell. This sequence of events could be confirmed by the loss of biosynthetic activity. DNA, RNA, and proteins synthesis were inhibited in *S. aureus* when treated with the flavonoids **1**, **2**, and **4**. A plausible explanation of this result is that, by damaging cytoplasm membrane, the compounds might interfere with the energetic metabolism depending on respiratory chain (*30*), since membrane potential is required for the active uptake of various metabolites and for the biosynthesis of macromolecules. The membrane potential is fundamental to the survival and growth of bacterial cells, it is essential for bacteria to maintain their capacity for ATP synthesis which is the main energy source for almost all chemical processes in living systems. Therefore, our data represents further evidence that, the loss of membrane potential might affect the overall bacterial metabolic activity, resulting in some biosynthetic pathway inhibition, as demonstrated by the strong inhibition of DNA, RNA, and protein synthesis. Previously, other flavonoids compounds (licochalcone A and a flavanone lonchocarpol A) were reported to inhibit the incorporation of radioactive precursors into macromolecules (*31*). Furthermore, the

licochalcones A and C were found to inhibit NADH-cytochrome c reductase in bacteria (*32*). As stated by O'Grady (1971) (*33*), the main problem in any investigation attempting to elucidate the mechanism of action of an antibacterial agent is that it is difficult to distinguish with certainty the primary event from those that follow. As for most of bactericidal antibiotic agents, a non specific mechanism could be postulated. Therefore we undertook to investigate if the action mode of these flavonoids compounds is bactericidal or bacteriostatic and to assess in lysis could be involved in the death of cells. As shown in Figures 6 and 7 compounds reduced rapidly the bacterial cell density and caused lysis of *S. aureus*. This observation suggested that the action of compounds **1**, **2**, and **4** in *S. aureus* could be bactericidal. Our result is supported by the observation that other flavonoids compounds such as epigallocatechin gallate and galangin induced 3-log reduction or more in viable counts of *S. aureus* (*11,34*). The rapid bactericidal activity of compounds **1**, **2**, and **4** suggested a non-specific action mechanism and that, they might damage bacterial membrane as demonstrated with other reference antibiotics such as daptomycin (*22*).

The toxicity information obtained from this study is useful in choosing doses for repeat-dose study. It has been reported that, flavonoids have low toxicity because they are widely distributed in edible plants and beverages, and have been used in medicine. When used as dietary supplements or as pure compounds in pharmacological doses, flavonoids do not appear to cause unwanted side effect. Even when raised to the level of 10% of total caloric intake, flavonoids supplementation has been shown non-toxic (*35,36*). Since the LD_{50} values are larger than 625 µg/g of larvae, we concluded that flavonoids compounds herein studied is likely to be nontoxic. Interestingly, previous study reported that compounds **1** and **3** were not toxic to normal human cell AML12 hepatocytes (*37*). In view of these observations, our compounds may be considered for preclinical studies for their use as antibacterial drug candidates.

Finally, this study has demonstrated that, flavonoids compounds studied have bactericidal/ bacteriolytic effects on *S. aureus*; DNA, RNA, and proteins synthesis are inhibited in the killing process and membrane permeabilization occurred. On the basis of these results we can suppose that the mechanism of action of these compounds is non-specific. Such information may assist in their optimisation as lead compound, considering their *in vivo* low toxicity evidence here reported. Taking into account the medical importance of MRSA strains, compounds **1**, **2**, and **4** can be considered as promising in the perspective of new antibiotic drugs discovery, with the possibility of making analogues with improved pharmacological or pharmaceutical properties.

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References

1. Chung PY, Navaratnam P, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. Ann Clin Microbiol Antimicrob. 2011; 10:25.
2. Zuo GY, Li Y, Wang T, Han J, Wang GC, Zhang YL, Pan WD. Synergistic antibacterial and antibiotic effects of bisbenzylisoquinoline alkaloids on clinical isolates of methicillin-resistant staphylococcus aureus (MRSA). Molecules. 2011; 16:9819-9826.
3. Rates SNK. Plants as source of drugs. Toxicon. 2001; 39:603-613.
4. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod. 2012; 75:311-335.
5. Adjanohoun JE, Aboubakar N, Dramane K, *et al*. Traditional medicine and pharmacopoeia: Contribution to ethnopharmacological and floristic studies in Cameroon. OAU/STRC, Lagos, Nigeria, 1996; p. 299.
6. Ngadjui BT, Abegaz BM, Dongo E, Tamboue H, Fogue K. Geranylated and prenylated flavonoids from the twigs of *Dorstenia mannii*. Phytochemistry. 1998; 48:349-354.
7. Ngadjui BT, Dongo E, Tamboue H, Fogue K, Abegaz BM. Prenylated flavanones from the twigs of *Dorstenia mannii*. Phytochemistry. 1999; 50:1401-1406.
8. Ngadjui BT, Kouam SF, Dongo E, Kapche GW, Abegaz BM. Prenylated flavonoids from the aerial parts of *Dorstenia mannii*. Phytochemistry. 2000; 55:915-919.
9. Abegaz BM, Ngadjui TB, Dongo E, Tamboue H. Prenylated chalcones and flavones from the leaves of *Dorstenia kameruniana*. Phytochemistry. 1998; 49:1147-1150.
10. Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000; 55:481-504.
11. Cushnie TTP, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. Int J Antimicrob Agents. 2011; 38:99-107.
12. Kuete V, Ngameni B, Tangmouo JG, Bolla JM, Alibert FS, Ngadjui TB, Pages JM. Efflux Pumps are involved in the defense of gram-negative bacteria against the natural products isobavachalcone and diospyrone. Antimicrob Agents Chemother. 2010; 54(5): 1749-1752.
13. Mbaveng AT, Ngameni B, Kuete V, Konga SI, Ambassa T, Roy R, Abegaz BM, Etoa FX, Ngadjui BT, Beng VP. Antimicrobial activity of the crude extracts and five flavonoids from the twigs of *Dorstenia barteri* (Moraceae). J Ethnopharmacol. 2008; 116:483-489.
14. Mbaveng AT, Kuete V, Ngameni B, Beng VP, Ngadjuim BT, Meyer JJ, Lall N. Antimicrobial activities of the methanol extract and compounds from the twigs of *Dorstenia mannii* (Moraceae). BMC Complement Altern Med. 2012; 12:83.
15. Ngadjui BT, Dongo E, Abegaz BM, Fotso S, Tamboue H. Dinklagins A , B and C: Three prenylated flavonoids and

other constituents from the twigs of *Dorstenia dinklagei*. Phytochemistry. 2002; 61:99-104.
16. Ngameni B, Ngadjui BT, Folefoc GN, Watchueng J, Abegaz BM. Diprenylated chalcones and other constituents from the twigs of *Dorstenia barteri* Var.*Subtriangularis*. Phytochemistry. 2004; 65:427-432.
17. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts. Third Informational Supplement. CLSI Document M27-S3. Clinical and Laboratory Standards Institute, Wayne, PA, USA. 2008.
18. Clinical and Laboratory Standards Institute. Reference method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard-8th Edition. CLSI Document M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2009.
19. Sims PJ, Waggoner AS, Wang CH, Hoffman JF. Studies on the mechanism by which cyanine dyes measure membrane potential in red blood cells and phosphatidylcholine vesicles. Biochemistry. 1974; 13: 3315-3330.
20. Ferrari ET, Widholm MJ. A simple, rapid, and sensitive method for estimation of DNA, RNA, and protein synthesis in carrot cell cultures. Anal Biochem. 1973; 56:346-352.
21. Paudel A, Hamamoto H, Kobayashi Y, Yokoshima S, Fukuyama T, Sekimizu K. Identification of novel deoxyribofuranosyl indole antimicrobial agents. J Antibiot. 2012; 65:53-57.
22. Ooi N, Miller K, Hobbs J, Rhys-Williams W, Love W, Chopra I. XF-73, a novel antistaphylococcal membrane-active agent with rapid bactericidal activity. J Antimicrob Chemother. 2009; 64:735-740.
23. Hamamoto H, Tonoike A, Narushima K, Horie R, Sekimizu K. Silkworm as a model animal to evaluate drug candidate toxicity and metabolism. Comp Biochem Physiol C Toxicol Pharmacol. 2009; 149:334-339.
24. Tsuchiya H, Sato M, Miyazaki T, *et al*. Comparative study on the antibacterial activity of phytochemical flavanones against methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol 1996; 50:27-34.
25. Sasaki H, Kashiwada Y, Shibata H, Takaishi Y. Prenylated flavonoids from *Desmodium caudatum* and evaluation of their anti-MRSA activity. Phytochemistry. 2012; 82:136-42.
26. Avila HP, Smania EDFA, Monache FD, Junior AS. Structure-activity relationship of antibacterial chalcones. Bioorg Med Chem. 2008; 16:9790-9794.
27. Patrzykat A, Friedrich LC, Zhang L, Mendoza V, Hancock EWR. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. Antimicrob Agents Chemother. 2002; 46:605-614.
28. Cushnie TTP, Lamb JA. Detection of galangin-induced cytoplasmic membrane damage in *Staphylococcus aureus* by measuring potassium loss. J Ethnopharmacol. 2005; 101:243-248.
29. Mirzoeva OK, Grishanin RN, Calder PC. Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria. Microbiol Res. 1997; 152:239-246.
30. Vinnikov AI, Oriabinskaia LB, Golodok LP. The effect of antibiotics on staphylococcal respiratory chain enzymes. Ukr Biokhim Zh. 1994, 66:51-58.
31. Mori A, Nishino C, Enoki N, Tawata S. Antibacterial activity and mode of action of plant flavonoids against *Proteus vulgaris* and *Staphylococcus aureus*. Phytochemistry. 1987; 26:2231-2234.
32. Haraguchi H, Tanimoto K, Tamura Y, Mizutani K, Kinoshita T. Mode of antibacterial action of retrochalcones from *Glycyrrhiza inflata*. Phytochemistry. 1998; 48:125-129.
33. O'Grady FW. Mode of action of antibacterial agents. Proc R Soc Med. 1971; 64:529-533.
34. Cushnie TTP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005; 26:343-356.
35. Middleton E, Kandaswami C. Effect of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol. 1992; 43:1167-1179.
36. Middleton JE, Kandaswami C. The impact of plant flavonoids on mammalian biology: Implications for immunity, inflammation and cancer. In Harbone JB (Ed). The flavonoids: Advances in research since 1986. Chapman & Hall, London, 1994; pp. 619-652.
37. Kuete V, Ngameni B, Wiench B, Krusche B, Horwedel C, Ngadjui BT, Efferth T. Cytotoxicity and mode of action of four naturally occuring flavonoids from the genus *Dorstenia*: Gancaonin Q, 4-hydroxylonchocarpin, 6-prenylapigenin, and 6,8-diprenyleriodictyol. Planta Med. 2011; 77:1984-989.

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