African Antivirulence Plants

Subjects: Plant Sciences | Microbiology

Contributor: Tsiry Rasamiravaka, Mondher El Jaziri

Antivirulence is the concept of blocking virulence factors produced by pathogenic organism. In regards to bacteria, the idea is to design agents that block virulence rather than kill bacteria population that generate more selective pressure leading to antibiotic resistance. African plants, through their huge biodiversity, present a considerable reservoir of secondary metabolites with a very broad spectrum of biological activities, a potential source of natural products targeting such non-microbicidal mechanisms.

Keywords: Virulence ; Biofilm ; medicinal plants ; natural compounds

1. Introduction

Antimicrobial resistance, increasingly observed within a wide range of pathogenic bacteria, has become a worldwide threat to public health [1][2]. Over time, bacteria adapt to the drugs that are designed to kill them, evolving or selecting resistance mechanisms to ensure survival. The resistance of bacteria to antibiotics is a naturally occurring phenomenon, supposedly progressive over contact with antibiotics. However, the misuse and abuse of antibiotics led to a strong and rapid selective pressure, leading to an uncontrolled widespread development of antibiotic-resistant bacteria [3][4]. Beyond resistance to antibiotics, the ability of bacteria to develop effective biofilms represents one of the major obstacles in the fight against bacterial infections. Indeed, while planktonic lifestyle allows bacteria to easily diffuse in diverse environments, their biofilm lifestyle allows efficient colonization of biotic and abiotic surfaces and protection from environmental aggression [5].

Undoubtedly, whenever new antimicrobial compounds would be discovered, their use will result in selective pressures, probably leading targeted bacteria to develop a resistance to these agents. This likely outcome stirs researchers to consider other strategies, notably based on the search for original compounds that impair virulence expression mechanisms and/or biofilm formation without affecting bacterial viability [6][7]. Striking such targets will likely impact invasion capabilities and aggressiveness of bacteria, as well as their ability to build protective barriers against host immune defenses or antibiotics; all the while, selective pressure would be minimized [8], most probably preventing or slowing down the apparition and spread of resistances.

The expression of bacterial virulence factors is generally coordinated by quorum sensing (QS) mechanisms, a cell-to-cell communication system that allows bacteria to detect their population density by producing and perceiving diffusible signal molecules that synchronize common behaviors [6]. Depending on bacteria species, QS regulates the production of virulence factors, motilities, and/or biofilm formation. Thus, the disruption of QS signaling, also termed quorum quenching (QQ), appears as interesting adjuvant strategies in the fight against bacterial infections [9].

Over millions of years of co-evolution, plants accumulated highly diverse secondary metabolites (so-called ‘natural products’), developing means of surviving in hostile environments that combine herbivorous insects and pathogenic bacteria, fungi, and viruses [10]. Given the huge diversity of flora and ecosystems in the world, the plants likely represent significant sources of innovative compounds with antivirulence properties. Indeed, several studies have already reported natural compounds, mainly isolated from plants, and synthetic compounds interfering with bacterial virulence [5][11]. For instance, ajoene, an allyl sulfide isolated from garlic (*Allium sativum* L., Liliaceae) and baicalin, a flavone glycoside isolated from Huangqin (*Scutellaria baicalensis* Georgi, Lamiaceae) have been reported to inhibit both virulence factors production and biofilm formation in *P. aeruginosa* through QQ pathways [12][13].

From an estimated African biodiversity of ~45,000 plant species, only 5000 have documented medicinal use [14]. The list of drugs provided by the African flora appears quite short (less than 100 active compounds) [15] compared with those from other traditional medical systems such as Traditional Chinese Medicine (more than 2000 active compounds from Chinese herbal) [16], suggesting an unrivalled opportunity for the discovery of new drugs.

2. African Plant Extracts with Antivirulence Activities

2.1. Activities on Gram-Negative Bacteria

Various South African medicinal plants have been widely investigated for their antivirulence activities against various Gram-negative bacteria:

(i) The hexanic extract from South African *Kigelia africana* (Lam.) Benth. (Fruits, Bignoniaceae) used to treat dysentery, reduces the production of violacein in *C. violaceum* ATCC 12472 by 65% when tested at 660 µg/mL [17]. Additionally, this extract interferes with the QS mechanism in *A. tumefaciens* by affecting the *luxI* synthase gene and the LuxR transcriptional regulator of autoinducers;

(ii) South African plants, used to treat urinary tract infections, have been studied by Baloyi et al. [18] for their effects on the production of violacein in *C. violaceum* ATCC 12472. At a final concentration of 330 µg/mL, the extracts of *Cenchrus ciliaris* L. (bark; methanol; Poaceae), *Cryptocarya latifolia* Sond. (bark; methanol; Lauraceae), *Eucomis autumnalis* (Mill.) Chitt. (bulb; methanol; Aspaagaceae), *Hydnora africana* Thumb. (bark; methanol; Aristolochiaceae), *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. (corme; dichlorométhane; Hypoxidaceae), *Rhoicissus tridentata* (L.f.) Wild & R.B. Drumm (root; methanol; Vitaceae), *Baccharoides adoensis* (Sch.Bip. ex Walp.) H.Rob. (synonym of *Vernonia adoensis* Sch.Bip. ex Walp.) (bark; aqueous; Asteraceae), *Bauhinia bowkeri* Harv. (root; aqueous; Fabaceae) inhibit the production of violacein in *C. violaceum* ATCC 12472 (from 4 to 43% inhibition);

(iii) Plants from the Myrtaceae family, endemic to South Africa and traditionally used to treat different ailments such as diarrhea, diabetes, reproductive problems, and respiratory diseases, have been investigated for their antibiofilm activity against various Gram-negative bacteria, including *P. aeruginosa* ATCC 27853, *Salmonella* ser. *typhimurium* ATCC 39183, and *E. coli* ATCC 25922 [19]. It has been highlighted that acetone extracts of leaves from different *Syzygium* and *Eugenia* species, particularly *S. legatii* Burtt Davy & Greenway and *E. erythrophylla* Strey, at 1000 µg/mL reduce biofilm formation (from 59–100% inhibition) but were unable to destroy pre-formed biofilms. These extracts exert in planktonic growth condition, a Minimal Inhibitory Concentration (MIC) values ranging from 40 to 310 µg/mL and relatively low cytotoxicity towards kidney epithelial cells (Vero) as concentrations of 140 to 1140 µg/mL inhibit cell viability by 50% (LC_{50}); this indicates potentially safe herbal products;

(iv) The ethanolic extract of the leaves of *Lessertia frutescences* (L.) Goldblatt & J.C.Manning (synonym of *Sutherlandia frutescens* (L) R.Br.) (Fabaceae), showed antibiofilm activity (90% inhibition) in *P. aeruginosa* ATCC 35032 and inhibition (>80%) of pyocyanin and LasB elastase [20].

Finally, (v) the methanolic extract of the South African plant *Sclerocarya birrea* (A.Rich.) Hoch. (trunk bark; Anacardiaceae) has been proposed to exhibit anti-QS activity as it inhibits the production of pyoverdine, protease and motility as well as biofilm formation (78% inhibition) in *P. aeruginosa* MTCC 2453 at the concentration of 100 µg/mL [21].

Plants from Western Africa, and particularly seven medicinal plants from Burkina Faso, have been also investigated for their impact on *C. violaceum* and *P. aeruginosa* QS mechanism and on *P. aeruginosa* biofilm formation; antivirulence activities of these Burkinabe plants are summarized in Table 1.

Table 1. Antivirulence activities of Burkinabe medicinal plants against Gram-negative bacteria.

Burkinabe Medicinal Plant	Plant Part	Tested Extract and Concentration	Production of Violacein in *C. violaceum* CV026	Production of Pyocyanin in *P. aeruginosa* PAO [1]	Production of Elastase in *P. aeruginosa* PAO1	Production of Biofilm in *P. aeruginosa* PAO [1]	References
Acacia dudgeoni **Craib. ex Holl.**	**Stem bark**	**Methanol 50–400 µg/mL [2,3]**	**−25% to −69%**	**−33% to −66%**	**NC**	**−25% to −59%**	[22]
Balanites aegyptiaca **(L.) Delille.**	**Leafy galls [1]**	**Methanol 100 µg/mL [2]**	**−10%**	**−15%**	**NC**	**−33%**	[23]
	Stem bark	**Methanol 100 µg/mL [2]**	**−15%**	**−20%**	**NC**	**−20%**	
Crossopteryx febrifuga **(Afzel ex G. Don) Benth**	**Leave and stem**	**Methanol 100 µg/mL [2]**	**NC**	**−52%**	**−48%**	**NC**	[24]

Burkinabe Medicinal Plant	Plant Part	Tested Extract and Concentration	Production of Violacein in *C. violaceum* CV026	Production of Pyocyanin in *P. aeruginosa* PAO [1]	Production of Elastase in *P. aeruginosa* PAO1	Production of Biofilm in *P. aeruginosa* PAO [1]	References
Terminalia leiocarpa **(DC.) Baill. [synonym of** ***Anogeissus leiocarpus*** **(DC) Guill. et Perr.]**	**Stem bark**	**Methanol 100 µg/mL [2]**	**−50%**	**−66%**	**NC**	**NC**	[25]
Terminalia macroptera **Guill. and Perr.**	**Stem bark**	**Methanol 100 µg/mL [2]**	**−35%**	**−50%**	**NC**	**−30%**	[23]
Vachellia seyal **(Delile) P.J.H.Hurter [synonym of** ***Acacia seyal*** **Delile]**	**Bark**	**Methanol 50–800 µg/mL [2,3]**	**−25% to −97%**	**−22% to −86%**	**−8% to −56%**	**At 800 µg/mL: −69%**	[26]
Zanthoxylum zanthoxyloides **(Lam) Zepern. and Timler**	**Stem bark**	**Methanol 100 µg/mL [2]**	**NC**	**−28%**	**−15%**	**NC**	[24]

[1] produced on leaves, following insect attack; [2] sub-inhibitory concentrations; [3] sub-inhibitory concentrations: <800 µg/mL.

These include *Acacia dudgeoni* Craib. ex Holl (Mimosaceae), used in the treatment of diarrhea and childhood dysentery [22][27]; *Balanites aegyptiaca* (L.) Delille (Zygophyllaceae) [23], traditionally used for the treatment of respiratory tract diseases, skin diseases, and wounds; *Crossopteryx febrifuga* (Afzel ex G. Don) Benth. (Rubiaceae) used in the treatment of various infectious diseases such as typhoid fever, respiratory infections, infected wounds, and dental diseases [24]; *Terminalia leiocarpa* (DC.) Baill. (synonym of *Anogeissus leiocarpus* (DC) Guill. et Perr.) (Combretaceae), also used to treat respiratory diseases and wounds [25]; *Terminalia macroptera* Guill. & Perr. (Combretaceae) [23], traditionally used for the treatment of respiratory tract diseases, skin diseases and wounds; *Vachellia seyal* (Delile) P.J.H.Hurter (synonym of *Acacia seyal* Delile) Del (Fabaceae) [26] traditionally used to treat toothache, dysentery, and burns [28] with reported potent antimicrobial activity [29]; and *Zanthoxylum zanthoxyloides* (Lam) Zepern. and Timler (Rutaceae) also used to treat typhoid fever, respiratory infections, infected wounds, and dental diseases [24].

Plants from Eastern Africa have been also investigated for their QQ properties in a *E. coli* model. About 25 medicinal plant extracts (roots, bark, and leaves; ethanol 50% v/v) used in southwestern Kenya to treat gastrointestinal and urinary tract infections were tested against a transformed *E. coli* Top 10 reporter QS strain that expresses green fluorescent protein (GFP) when induced by exogenous AHLs (3-oxo-C6HSL). Interestingly, the extracts of *Vachellia gerrardii* (Benth.) P.J.H.Hurter (synonym of *Acacia gerrardii* Benth) and *Elaeodendron buchananii* (Loes.) Loes. barks, at 1000 µg/mL reduce the reporter GFP expression (up to 50% inhibition) without any effect on the *E. coli* biofilm formation [30]. In the same line, two Ethiopian antimicrobial medicinal plants have been reported to exert anti-QS without affecting bacterial viability; the root methanolic extract of *Albizia schimperiana* Oliv. (Fabaceae) and seed petroleum ether extract of *Justicia schimperiana* (Hochst. ex Nees) T. Anderson (Acanthaceae) at 6500 µg/mL interfere with cell-to-cell communication, most likely by interacting with the 3-oxo-C6-HSL signaling pathway in *E. coli* reporter strain AI1-QQ.1 [31].

For almost 10 years, antivirulence activities of endemic plants from Madagascar have been regularly reported. Indeed, the bark of *D. pervillei* Vakte and *D. trichocarpa* Baker (Fabaceae; hexane extracts tested at 300 µg/mL) inhibit the production of *P. aeruginosa* virulence factors pyocyanin and elastase by 43% and 25%, respectively, and the expression of QS-related (*lasI*, *lasR*, *rhlI*, and *rhlR*) and QS-regulated (*lasB* and *rhlA*) genes [32]. Further investigations on the hexane bark extract of *D. trichocarpa,* traditionally used to treat various ailments—such as laryngitis, diarrhea, and rheumatic pains—led to the generation of active fractions which exert anti-QS and/or antibiofilm activities. Particularly, an active fraction exerts antibiofilm activities at 200 µg/mL (63% inhibition) without affecting bacterial viability or QS mechanism of *P. aeruginosa* PAO1. The inhibition of biofilm formation is probably linked to a reduction in flagellar-dependent motilities (swimming and swarming) as well as in exopolysaccharides production [33]. In Madagascar, a mixture of *Tephrosia purpurea* L. (Fabaceae) and *Buddleja madagascariensis* Lam (Buddlejaceae) macerated in cow dung manure is traditionally used as a phytotreatment against potato crops diseases such as potato leaf spots caused by the phytophatogen *R. solanacearum* [34]. The study of their antibacterial effects demonstrated that the methanolic extracts of

both plants, tested at 100 µg/mL, reduce the expression of *P. aeruginosa* PAO1 QS-regulated *lasB* (45% and 52% inhibition, respectively) and *rhlA* (32% and 33% inhibition, respectively) genes; but only *T. purpurea* extracts exhibit antibiofilm activities against *P. aeruginosa* PAO1 and *R. solanacearum* (52% and 30% inhibition, respectively) without affecting bacterial growth [35].

Finally, the dichloromethane extract of *Cordia gilletii* De Wild (Boraginaceae) root barks, medicinal plant from the Democratic Republic of Congo, a plant known for bactericidal activities against pathogenic microorganisms such as *E. coli* [36], was also found to quench the production of pyocyanin, to inhibit the expression of several QS-regulated genes (i.e., *lasB, rhlA, lasI, lasR, rhlI,* and *rhlR;* 35%, 40%, 24%, 25%, 52%, and 23% inhibition, respectively) and to reduce biofilm formation (21% inhibition) in *P. aeruginosa* PAO1 without affecting its viability [37].

2.2. Activities on Gram-Positive Bacteria

To date, only South African plants have been investigated for their antivirulence activities against Gram-positive bacteria:

(i) the extracts (concentration, 330 µg/mL) from *Cenchrus ciliaris* L. (bark; methanol; Poaceae), *Eucomis autumnalis* (Mill.) Chitt. (bulb; methanol; Asparagaceae), *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. (corme; dichloromethane; Hypoxidaceae), *Bacharoides adoensis* (Sch.Bip. ex Walp.) H.Rob. [synonym of *Vernonia adoensis* Sch. Bip. ex Walp.] (bark; water; Asteraceae), which are all used to treat urinary tract infections, inhibit biofilm formation (by 21–38%) in *S. aureus* ATCC 25923 [18];

(ii) the acetone leaves extracts of different *Syzygium* and *Eugenia* species at 1000 µg/mL present antibiofilm activities against *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, and *B. cereus* ATCC 21366 [17]. Particularly, the extract of *S. gerrardii* (Harv. ex Hook.f.) Burtt Davy had the capacity to reduce biofilm formation in all tested strains (68–100% inhibition) and to destroy pre-formed biofilms (69–100% biofilm dispersion);

(iii) the dichloromethane/methanol extracts of plants traditionally used to treat oral infections, including *Vachellia karroo* (Hyane) Banfi & Galasso (synonym of *Acacia karroo* Hayne) (leaves; Fabaceae), *Erythrina lysistemon* Hutch (bark; Fabaceae), *Spyrostachys africana* Sond. (leaves; Euphorbiaceae), *Tecoma capensis* (Thumb.) Lindl., (leaves; Bignoniaceae) and *Tarchonanthus camphoratus* L. (leaves; Asteraceae), at a concentration of 250 µg/mL, showed antibiofilm activity against *S. mutans* ATCC 25175, known to be responsible for dental caries, with 59%, 68%, 86%, 52%, and 54% inhibition, respectively [38];

(iv) when the *L. monocytogenes* ATCC 19111 is grown in the presence of 1000 µg/mL of dichloromethane/methanol leaves extract of *Agathosma betulina* (P.J. Bergius) Pillans, (Rutaceae), and *Aspalathus linearis* (Burm.f.) R. Dahlgren (Fabaceae), plants exclusively found in South Africa, biofilm formation reduction was observed with an inhibition rate of 20% and 75%, respectively [39].

3. Compounds isolated from African plants with Antivirulence Activities

3.1 Activities on Gram negative bacteria

Several active fractions containing flavonoid-like compounds, obtained from the bark of *Combretum albiflorum* (Tul.) Jongkind, (Combretaceae), a Madagascar endemic plant [40], were found to inhibit the production of QS-regulated extracellular virulence factors (violacein in *C. violaceum* CV026 and pyocyanin in *P. aeruginosa* PAO1) [41]. Among these flavonoids, the flavan-3-ol catechin, at 4 mM in *P. aeruginosa* PAO1, had a negative impact on the production of violacein (75 % inhibition), pyocyanin (50% inhibition), elastase (30 % of inhibition), on biofilm formation (30 % inhibition) and on the transcription of several QS-related genes (i.e., *lasI, lasR, rhlI, rhlR, lasB,* and *rhlA*). Synthetic epicatechin reduces the *P. aeruginosa* production of pyocyanin (50 % inhibition) and elastase (30 % inhibition) without effect on biofilm formation. By contrast, epicatechin isolated from *Ficus sansibarica* Warb.(Moraceae), collected in KwaZulu-Natal, South Africa,reduces the adhesion to polystyrene surfaces of Gram negative *E. coli* ATCC 25922 up to 15 % at 3.4 mM [42].

The methyl gallate (MG) isolated from the galls (produced on leaves following insect attack) methanol extract of *Guiera senegalensis* J. F. Gmel (Combretaceae), a traditional burkinabe treatment of cough, dysentery and malaria, has been shown to exert antivirulence activities [43], Methyl gallate presents MIC values of 512 and 64 µg/mL against *P. aeruginosa* PAO1 and *C. violaceum* CV026, respectively, but, at 12.5 µg/mL (67.9 µM), already inhibits the production of pyocyanin (by 65 %) and violacein (by 10 %). These antivirulence activities are in correlation with the data of Hossain et al. [44] who showed that the production of pyocyanin was inhibited (37 - 64 %) by MG in a concentration-dependent manner (16 - 256 µg/mL). Moreover, in *P. aeruginosa* PAO1, MG reduces the expression of the AHL synthetases genes (*lasI* and *rhlI*) and the QS regulator genes (*lasR* and *rhlR*) and biofilm formation.

A triterpenoid coumarate ester has been isolated from *Dalbergia trichocarpa* Baker(Malagasy endemic species)barkextract as a major bioactive compound. Indeed, oleanolic aldehyde coumarate (OALC), at 200 µM, inhibits the formation of *P. aeruginosa* PAO1 biofilm (by 44 %) and its maintenance as well as the expression of the *las* and *rhl* QS systems [5]. As a consequence, the production of QS-controlled virulence factors, including rhamnolipids, pyocyanin, elastase and extracellular polysaccharides, as well as twitching and swarming motilities are significantly reduced (75 %, 64 %, 19 %, 44 %, 40 % and 52 % inhibition, respectively). Additionally, OALC disorganizes established biofilm structure and significantly increases the bactericidal activity of tobramycin against biofilm-encapsulated PAO1 cells. Consistently, *in vivo* experiments indicated that OALC treatment reduces *P. aeruginosa* pathogenicity in *Caenorhabditis elegans*, a nematode.

The monocyclic diterpenoid cassipourol and the phytosterol β-sitosterol, isolated from *Platostoma rotundifolium* (Briq.) A. J. Paton (Lamiaceae), a Burundian anti-infectious plant [45], inhibit QS-regulated and QS-regulatory genes expression in *las* and *rhl* systems and disrupt the formation of biofilms by *P. aeruginosa* at concentrations down to 12.5 and 50 µM, respectively [46]. Authors also isolated α-amyrin, a biosynthesis precursor of ursolic acid [47], that exerts antibiofilm properties at 50 µM without any effect on QS-regulatory genes expression; this suggests that other ursane and oleane-type triterpenes may exert antibiofilm properties with similar mechanisms of action. The three isolated compounds improve swimming but not twitching motilities which consequently promotes planktonic lifestyle in *P. aeruginosa* PAO1 and dispersal on preformed biofilms. Interestingly, the addition of cassipourol, α-amyrin and β-sitosterol (100 µM) considerably improved the effectiveness of tobramycin (50 µg/mL = 107 µM) against *P. aeruginosa* PA01 with a drastic reduction in cell viability of biofilm-encapsulated bacteria (89 %, 70 % and 76 % of bacterial death, respectively, versus 40 % in DMSO control treatment).

4. Discussion

African plants screened so far provide a clear indication that we have a fairly large source of non-microbicidal natural products active on bacteria. According to literature, the search for antivirulence activities have been shyly initiated since the last decade; by contrast, there has been a wide research on conventional antimicrobial activities (i.e, bactericidal activity) of African plants over the past thirty years [48][49][50]. Although African plants investigated for antivirulence activities are diverse (largely from Southern and Eastern African regions), very few studies have resulted in the characterization of the active compound(s), suggesting that this investigation is only beginning, which highlights a huge potential for new substances still to be discovered. So far however, it should be acknowledged that the discovery of QS modulators has not yet led to major therapeutic breakthroughs; also, QS systems do not control the totality of virulence factors expression and the development of anti-QS bacterial resistance cannot be excluded. But this should not prevent further research in this promising field. Indeed, according to in vitro experiments, the combination of antibiotics and antivirulence (e.g. cassipourol) agents has already demonstrated its potential and about 33 compounds or agents, mainly from synthetic origin, that target virulence factors are under way for preclinical investigations, most of them focusing on *P. aeruginosa, Enterobacteriaceae* spp. and *S. aureus*.

References

1. WHO. World Health Organization Releases GLOBAL priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics. J. Med. Soc. 2017, 32, 76–77.
2. Álvarez-Martínez, F.J.; Barrajón-Catalán, E.; Micol, V. Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. Biomedicines 2020, 8, 405.
3. Andersson, D.I.; Hughes, D. Antibiotic Resistance and Its Cost: Is It Possible to Reverse Resistance? Nat. Rev. Microbiol. 2010, 8, 260–271.
4. Spellberg, B.; Talbot, G. Recommended Design Features of Future Clinical Trials of Antibacterial Agents for Hospital-Acquired Bacterial Pneumonia and Ventilator-Associated Bacterial Pneumonia. Clin. Infect. Dis. 2010, 51, 150–170.
5. Rasamiravaka, T.; Vandeputte, O.M.; Pottier, L.; Huet, J.; Rabemanantsoa, C.; Kiendrebeogo, M.; Andriantsimavandy, A.; Rasamindrakotroka, A.; Stévigny, C.; Duez, P.; et al. Pseudomonas aeruginosa Biofilm Formation and Persistence, along with the Production of Quorum Sensing-Dependent Virulence Factors, Are Disrupted by a Triterpenoid Coumarate Ester Isolated from Dalbergia trichocarpa, a Tropical Legume. PLoS ONE 2015, 10, e0132791.
6. Carette, J.; Nachtergael, A.; Duez, P.; El Jaziri, M.; Rasamiravaka, T. Natural Compounds Inhibiting Pseudomonas aeruginosa Biofilm Formation by Targeting Quorum Sensing Circuitry. In Bacterial Biofilms; Intechopen: London, UK, 2020.

7. Dickey, S.W.; Cheung, G.Y.C.; Otto, M. Different Drugs for Bad Bugs: Antivirulence Strategies in the Age of Antibiotic Resistance. Nat. Rev. Drug Discov. 2017, 16, 457–471.
8. Ogawara, H. Possible Drugs for the Treatment of Bacterial Infections in the Future: Anti-Virulence Drugs. J. Antibiot. 2020, 1–18.
9. Mion, S.; Rémy, B.; Plener, L.; Chabrière, É.; Daudé, D. Quorum Sensing and Quorum Quenching: How to Disrupt Bacterial Communication to Inhibit Virulence? Med. Sci. 2019, 35, 31–38.
10. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. Am. J. Clin. Nutr. 2004, 79, 727–747.
11. Song, X.; Xia, Y.X.; He, Z.D.; Zhang, H.J. A Review of Natural Product with Anti-Biofilm Activity. Curr. Org. Chem. 2018, 22, 789–817.
12. Jakobsen, T.H.; van Gennip, M.; Phipps, R.K. Ajoene, a Sulfur-Rich Molecule from Garlic, Inhibits Genes Controlled by Quorum Sensing. Antimicrob. Agents Chemother. 2012, 56, 2314–2325.
13. Luo, J.; Dong, B.; Wang, K.; Cai, S.; Liu, T.; Cheng, X.; Chen, Y. Baicalin Inhibits Biofilm Formation, Attenuates the Quorum Sensing-Controlled Virulence and Enhances Pseudomonas aeruginosa Clearance in a Mouse Peritoneal Implant Infection Model. PLoS ONE 2017, 12, e0176883.
14. Mahomoodally, M.F. Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants. Evid. Based Complement. Altern. Med. 2013, 2013, 1–14.
15. Kahumba, J.; Rasamiravaka, T.; Okusa, P.N.; Bakari, A.S.; Bizumukama, L.; Kiendrebeogo, M.; El Jaziri, M.; Williamson, E.M.; Duez, P. Traditional African Medicine: From Ancestral Know-How to Bright Future. Science 2015, 350, S61–S63.
16. Ehrman, T.M.; Barlow, D.J.; Hylands, P.J. Phytochemical Databases of Chinese Herbal Constituents and Bioactive Plant Compounds with Known Target Specificities. J. Chem. Inf. Model. 2007, 47, 254–263.
17. Chenia, H.Y. Anti-Quorum Sensing Potential of Crude Kigelia africana Fruit Extracts. Sensors 2013, 13, 2802–2817.
18. Baloyi, I.T.; Cosa, S.; Combrinck, S.; Leonard, C.M.; Viljoen, A.M. Anti-Quorum Sensing and Antimicrobial Activities of South African Medicinal Plants against Uropathogens. S. Afr. J. Bot. 2019, 122, 484–491.
19. Famuyide, I.M.; Aro, A.O.; Fasina, F.O.; Eloff, J.N.; McGaw, L.J. Antibacterial and Antibiofilm Activity of Acetone Leaf Extracts of Nine Under-Investigated South African Eugenia and Syzygium (Myrtaceae) Species and Their Selectivity Indices. BMC Complement. Altern. Med. 2019, 19, 141.
20. Cosa, S.; Chenia, H. Investigating the Potential of Ten South African Indegenious Plants Extracts as Quorum Sensing Inhibitors (QSI) of Pseudomonas aeruginosa Virulence-Related Factors. J. Biotechnol. Biomater. 2014, 3, 112.
21. Sarkar, R.; Chaudhary, S.K.; Sharma, A.; Yadav, K.K.; Nema, N.K.; Sekhoacha, M.; Karmakar, S.; Braga, F.C.; Matsabisa, G.; Mukherjee, P.K.; et al. Anti-Biofilm Activity of Marula—A Study with the Standardized Bark Extract. J. Ethnopharmacol. 2014, 154, 170–175.
22. Ouedraogo, V.; Karama, I.; Rouamba, A.; Compaoré, M.; Kiendrebeogo, M. Acacia dudgeoni Craib. Ex Holl (Mimosaceae): Potential Inhibitor of Biofilm Formation and Quorum Sensing in P. aeruginosa PAO1. Int. J. Biotechnol. 2019, 23, 1–6.
23. Ouedraogo, V.; Rouamba, A.; Compaoré, E.; Compaoré, M.; Kiendrebeogo, M. Antioxidant, Antiquorum-Sensing and Antibiofilm Activities of Balanites aegyptiaca (L.) Del. (Balanitaceae) and Terminalia macroptera Guill. and Perr. (Combretaceae). Adv. Biochem. 2018, 6, 26–31.
24. Ouedraogo, V.; Compaoré, E.; Rouamba, A.; Compaoré, M.; Kiendrebeogo, M. Anti-Virulence Activity of Three Medicinal Plants: Cassia occidentalis L., Crossopteryx febrifuga (Afzel Ex G. Don) Benth. and Zanthoxylum zanthoxyloides (Lam) Zep. and Timl. Int. J. Microbiol. Res. 2019, 29, 1–7.
25. Ouedraogo, V.; Kiendrebeogo, M. Methanol Extract from Anogeissus leiocarpus (DC) Guill. et Perr. (Combretaceae) Stem Bark Quenches the Quorum Sensing of Pseudomonas aeruginosa PAO1. Medicines 2016, 3, 26.
26. Ouedraogo, V.; Rouamba, A.; Compaore, M.; Sombié, P.A.E.D.; Kiendrebeogo, M. Acacia seyal Del Bark Extract Reduces Quorum Sensing—Controlled Virulence Factor Production and Biofilm Formation in Pseudomonas aeruginosa PAO1. Int. J. Curr. Res. Biosci. 2018, 5, 7–13.
27. Bangré, Y.A.; Mètuor-dabiré, A.; Rouamba, A.; Kiendrebeogo, M.; Simporé, J. Antibacterial Activity of Methanol Bark Extract from Acacia dudgeoni craib. Ex Holl (Mimosaceae) on Growth of Cefotaxime Resistant Escherichia coli. Curr. Res. Microbiol. Biotechnol. 2018, 6, 1660–1664.
28. Sereme, A.; Millogo-Rasolodimby, J.; Guinko, S.; Nacro, M. Proprietes Therapeutiques des Plantes a Tanins Du Burkina Faso (Therapeutic Power of Tannins Producing Species of Burkina Faso). Afr. J. Tradit. Med. 2008, 15, 41–49.

29. Alawi, S.M.A.; Hossain, M.A.; Abusham, A.A. Antimicrobial and Cytotoxic Comparative Study of Different Extracts of Omani and Sudanese Gum acacia. J. Basic Appl. Sci. 2018, 7, 22–26.
30. Omwenga, E.O.; Hensel, A.; Pereira, S.; Shitandi, A.A.; Goycoolea, F.M. Antiquorum Sensing, Antibiofilm Formation and Cytotoxicity Activity of Commonly Used Medicinal Plants by Inhabitants of Borabu Sub-County, Nyamira County, Kenya. PLoS ONE 2017, 12, e0185722.
31. Bacha, K.; Tariku, Y.; Gebreyesus, F.; Zerihun, S.; Mohammed, A.; Weiland-Bräuer, N.; Schmitz, R.A.; Mulat, M. Antimicrobial and Anti-Quorum Sensing Activities of Selected Medicinal Plants of Ethiopia: Implication for Development of Potent Antimicrobial Agents. BMC Microbiol. 2016, 16, 139.
32. Rasamiravaka, T.; Jedrzejowski, A.; Kiendrebeogo, M.; Rajaonson, S.; Randriamampionona, D.; Rabemanantsoa, C.; Andriantsimavandy, A.; Rasamindrakotroka, A.; Duez, P.; El Jaziri, M.; et al. Endemic Malagasy Dalbergia species Inhibit Quorum Sensing in Pseudomonas aeruginosa PAO1. Microbiology 2013, 159, 924–938.
33. Rasamiravaka, T.; Quentin, L.; Mol, A.; Megalizzi, V.; Rabemanantsoa, C.; Duez, P.; El Jaziri, M. An Active Fraction from Dalbergia trichocarpa Baker Disrupts the Formation and Maintenance of Biofilms in Pseudomonas aeruginosa PAO1. IARJSET 2016, 3, 124–133.
34. Rasamiravaka, T.; Rajaonarivelo, J.P.; Rabemanantsoa, C.; El Jaziri, M.; Andrianarisoa, B.; Duez, P. Malagasy Traditional Treatments for Food Crops: A Tool to Control Potato Bacterial Diseases? Crop. Prot. 2017, 102, 49–55.
35. Rasamiravaka, T.; Raveloson, P.A.; Rajaonarivelo, P.J.; Rabemanantsoa, C.; Andrianarisoa, B.; Duez, P.; El Jaziri, M. Malagasy Traditional Treatments of Infectious Plant Diseases Exert Anti-Virulence Activities Against Pseudomonas aeruginosa and Ralstonia solanacearum. J. Microbiol. Biotechnol. Food Sci. 2018, 7, 377–382.
36. Okusa, P.N.; Penge, O.; Devleeschouwer, M.; Duez, P. Direct and Indirect Antimicrobial Effects and Antioxidant Activity of Cordia gilletii De Wild (Boraginaceae). J. Ethnopharmacol. 2007, 112, 476–481.
37. Okusa, P.; Rasamiravaka, T.; Vandeputte, O.; Stvigny, C.; El Jaziri, M.; Duez, P. Extracts of Cordia gilletii De Wild (Boraginaceae) Quench the Quorum Sensing of Pseudomonas aeruginosa PAO1. J. Intercult. Ethnopharmacol. 2014, 3, 138.
38. Akhalwaya, S.; van Vuuren, S.; Patel, M. An In Vitro Investigation of Indigenous South African Medicinal Plants Used to Treat Oral Infections. J. Ethnopharmacol. 2018, 210, 359–371.
39. Sandasi, M.; Leonard, C.M.; Viljoen, A.M. The In Vitro Antibiofilm Activity of Selected Culinary Herbs and Medicinal Plants against Listeria monocytogenes. Lett. Appl. Microbiol. 2010, 50, 30–35.
40. Jongkind, C.C.H. Prodromus for a Revision of Combretum (Combretaceae) for Madagascar. Bull. Mus. Natl. Hist. Nat. 1995, 17, 191–200.
41. Hossain, M.A.; Lee, S.J.; Park, N.H.; Mechesso, A.F.; Birhanu, B.T.; Kang, J.; Reza, M.A.; Suh, J.W.; Park, S.C. Impact of Phenolic Compounds in the Acyl Homoserine Lactone-Mediated Quorum Sensing Regulatory Pathways. Sci. Rep. 2017, 7, 1–16.
42. Ngezahayo, J.; Havyarimana, F.; Hari, L.; Stévigny, C.; Duez, P. Medicinal Plants Used by Burundian Traditional Healers for the Treatment of Microbial Diseases. J. Ethnopharmacol. 2015, 173, 338–351.
43. Babalola, I.T.; Shode, F.O. Ubiquitous Ursolic Acid: A Potential Pentacyclic Triterpene Natural Product. J. Pharmacogn. Phytochem. 2013, 2, 214–222.
44. Khameneh, B.; Iranshahy, M.; Soheili, V.; Fazly Bazzaz, B.S. Review on Plant Antimicrobials: A Mechanistic Viewpoint. Antimicrob. Resist. Infect. Control 2019, 8, 2–28.
45. Madureira, A.M.; Ramalhete, C.; Mulhovo, S.; Duarte, A.; Ferreira, M.J.U. Antibacterial Activity of Some African Medicinal Plants Used Traditionally Against Infectious Diseases. Pharm. Biol. 2012, 50, 481–489.
46. Van Vuuren, S.F. Antimicrobial Activity of South African Medicinal Plants. J. Ethnopharmacol. 2008, 119, 462–472.
47. Ngezahayo, J.; Pottier, L.; Ribeiro, S.O.; Delporte, C.; Fontaine, V.; Hari, L.; Stévigny, C.; Duez, P. Plastotoma rotundifolium Aerial Tissue Extract Has Antibacterial Activities. Ind. Crops Prod. 2016, 86, 301–310.
48. Strugeon, E.; Tilloy, V.; Ploy, M.-C.; Da Re, S. The Stringent Response Promotes Antibiotic Resistance Dissemination by Regulating Integron Integrase Expression in Biofilms. mBio 2016, 7, 1–9.
49. Mellbye, B.; Schuster, M. The Sociomicrobiology of Antivirulence Drug Resistance: A Proof of Concept. mBio 2011, 2, 1–4.
50. Parrino, B.; Schillaci, D.; Carnevale, I.; Giovannetti, E.; Diana, P.; Cirrincione, G.; Cascioferro, S. Synthetic Small Molecules as Anti-Biofilm Agents in the Struggle against Antibiotic Resistance. Eur. J. Med. Chem. 2019, 161, 154–178.