Anthraquinones as potential antimicrobial agents - A review

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Ethnopharmacological studies on antimicrobial traditional herbal preparations have gained much attention in recent years. In fact, in the last 30 years, 2/3 of the new antibacterial drugs were of natural source and among them, plants were mainly used. In addition, the emergence of new drugs active against resistant strains (e.g. the ESKAPE pathogens) to the actual chemotherapy, reinforces the interest of the prosecution of the studies concerning the discovery of new antibiotics from natural sources. Anthraquinones and their derivatives are a class of aromatic compounds with a 9,10-dioxoanthracene core. Considering the great structural diversity and variations in chemical composition, numerous antimicrobial *in vitro* and/or *in vivo* activities of natural and synthetic anthraquinones have been reported, however there has been limited research related to the structural-functional relationship of these compounds. The present study aimed to provide a comprehensive review of the available literature on chemical structure, mechanism of action and safety of anthraquinones as promising sources of antimicrobial lead-compounds.

Keywords: Anthraquinone derivatives; antimicrobial activity; emodin; natural sources

1. Introduction

Antimicrobial resistance (AMR) is a growing global healthcare problem due to the loss of efficacy of first line antibiotics. Many pathogens are developing resistance to multiple drugs, some to nearly all [1]. The major resistance overall issues being related to the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), specially to methicillin-resistant *Staphylococcus aureus* (MRSA), extended spectrum *β*-lactamase (ESBL) producing Enterobacteriaceae, fluoroquinolone-resistant (FQR) Gram-negative bacteria, multidrug-resistant (MDR) *Pseudomonas aeruginosa*, and the emerging vancomycin-resistant enterococci (VRE) [2].

The emergence of these new pathogens caused worldwide agencies to unite efforts in the discovery and development (D&D) of new antimicrobial drugs [3-4].

The first step of drug D&D is lead discovery, for which academia has made important contributions in the past (e.g. penicillin, streptomycin). Only a small fraction of marine, fungal and plant resources have been chemically and pharmacologically investigated, although nature still offers a high potential for drug lead discovery notably among anti-infective compounds [5]. In fact, in the last 30 years, 2/3 of the new antibacterial drugs were of natural source and among them, plants were mainly used [6].

Several plant-derived chemical compounds have been studied with this aim, including alkaloids, flavonoids, tannins, quinones, volatile terpenoids and phenol acids and other secondary metabolites. Among them, anthraquinone derivatives (AQ) have aroused special interest since they have demonstrated potential therapeutic uses as antibacterial, antiviral, antifungal as well as antioxidant, anti-inflammatory and cytotoxic agents[7]. Both natural and synthetic anthraquinones have now widespread application throughout industry and medicine. Important anthraquinone-bearing plant families are the Caesalpiniaceae, Polygonaceae, Rhamnaceae and Rubiaceae, and this class of compounds became marker chemical classes with chemotaxonomic significance to these plant families [8].

Anthraquinones and their derivatives are a class of aromatic compounds with a 9,10-dioxoanthracene. Although they exhibit great structural diversity and variations in chemical composition, they are divided into two main types: alizarin and emodin. The alizarin type is used as natural dye in the textile industry, while the emodin type was formerly used as laxative agent.

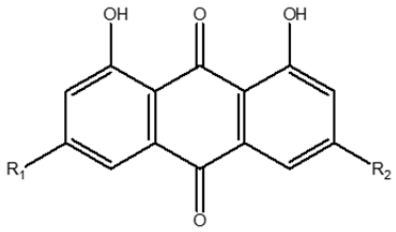
The *in vitro* antimicrobial activity of the emodin type anthraquinone compounds have been reported in many different studies [9]. The chemical structures of the main antimicrobial anthraquinones of this type are shown in Fig.1.

For instance:

- Aloe-emodin, a typical *Aloe* species anthraquinone was showed to inhibit Herpes simplex viruses (HSV-1, HSV-2), varicella-zoster virus, pseudorabies virus, influenza virus, MRSA, *Aspergillus fumigatus*, *Bacillus subtilis Candida albicans*, *Cryptococcus neoformans*, *Helicobacter pylori* and *Trichophyton mentagrophytes*;
- chrysophanol, a constituent of *Rheum* species, inhibited *A. fumigatus*, *C. albicans*, *C. neoformans*, *T. mentagrophytes*, Poliovirus type 2 and type 3;
- 8–dihydroxyanthraquinone, a marker compound of *Senna occidentalis*, inhibited *Clostridium perfringens* and *Staphylococcus aureus*;
- Emodin, found in several species and particularly in *Rhamnus frangula*, inhibited MRSA, cytomegalovirus (CMV), HSV-1, HSV-2, *B. subtilis*, *Helicobacter pylori*, *Heterobasidion annosum*, *Leishmania donovani*, *Plasmodium falciparum* and *Trypanosoma* spp.;

- Hypericin, an anthraquinone derivative found in *Hypericum perforatum*, inhibited HSV-1 and HSV-2, human immunodeficiency virus, human papillomavirus (HPV-1, HPV-6, HPV-11, HPV-16, and HPV-18), methicillin sensitive *Staphylococcus (*MSSA), MRSA, *H. pylori*, *B. subtilis* and *Bacillus cereus*;
- Physcion, also a typical constituent of *Rheum* species inhibited HSV-1 and HSV-2, CMV, *C. albicans*, *C. neoformans*, *T. mentagrophytes*, and *Aspergillus fumigatus*;
- Rhein inhibited *A. fumigatus*, *Bacteroides fragilis*, *B. subtilis*, *C. albicans*, *C. neoformans*, *H. pylori*, MRSA, *Neisseria gonorrhoeae* and *Streptococcus viridans* and *T. mentagrophytes* [10].

Hereby a brief update about anthraquinones antimicrobial activity, given an overview on their mechanism of action and their structural-functional studies will be presented.



	R1	R2
Aloe-emodin	H	CH_2OH
Emodin	OH	CH_3
Rhein	H	COOH
Physcione	CH_3	OCH_3
Chrysophanol	H	CH_3

Fig. 1 Principal emodin type anthraquinone natural products

2. Reported antimicrobial activities of anthraquinones

The antimicrobial activities of anthraquinones have been extensively studied *in vitro* on pure compounds or in crude plant extracts containing these class of constituents as marker compounds. Most of them exhibit positive activity against a large panel of reference of the most common pathogens, including the main causative agents of currently no treatable infections. Details of representative studies will be presented in this section.

The methanolic extract of the root of *Colubrina greggii* showed antimicrobial activity against *B. subtilis* and *S. aureus*. The bioassay-guided purification of the organic crude extract resulted in the isolation and identification of chrysophanol (1,8-dihydroxy-3-methylanthracenedione), as the metabolite responsible for the antimicrobial activity of the extract [11].

The minimum inhibitory concentration (MIC) value of *Aloe vera* gel and *Aloe vera* juice were evaluated against *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC10418, *Enterococcus faecalis* ATCC29212, *Salmonella typhimurium* ATCC29922, *Staphylococcus aureus* ATCC6571, *Staphylococcus epidermididis* ATCC29213, *Proteus vulgaris* ATCC13315 and *Pseudomonas aeruginosa* ATCC1062. *Aloe vera* juice showed an inhibitory effect against all the microorganisms but *Aloe vera* gel was only effective against *S. aureus* (10.54± 0.43 mm). In sequence, MIC was determined for the *Aloe vera* juice having it shown to be especially active against *Proteus vulgaris*. It could be theorized that presence of greater amount of the anthraquinones in the extract could be responsible for the high and broad spectrum antimicrobial activity of the juice as compared to gel [12], although *Aloe vera* inner gel (containg emodin, aloe-emodin, chrysophanol, rhein and physcion) expressed antibacterial properties against both susceptible and resistant *Helicobacter pylori* strains (MIC: 6.25-400 μg/mL). which may impact on the antimicrobial resistance phenomenon of *H. pylori*, proposing the *A. vera* inner gel as a novel effective natural agent in combination with antibiotics for the treatment of *H. pylori* gastric infection [13].

Antibacterial activity of a crude extract from *Rheum rhabarbarum* and its major bioactive compounds (aloe-emodin, rhein, emodin, chrysophanol, and physcion) was evaluated against *Aeromonas hydrophila* (Gram-negative, rod-shaped bacterium). The antibacterial activity given by MIC was positively related to the anthraquinone content ($r = 0.9306$, $P<0.01$) and the MIC values of the five isolated anthraquinones against *A. hydrophila* were found to be in the range 50–200 μg/mL [14].

In another study regarding several species of the same genus, the crude ethanol extracts obtained from the rhizome and roots of *Rheum palmatum*, *Rheum undulatum* and *Rheum rhaponticum*. showed a higher activity against reference strains of Gram-positive bacteria (*Staphylococcus* spp.) than against Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*). The strongest inhibitory effect against *Staphylococcus* spp. was exerted by *R. undulatum* extract (MIC = 125-250 μg/mL) and it was found that the active constituents were anthraquinones derivatives, including aloe-emodin, emodin and rhein. The moderate *in vitro* antibacterial activity of *R. undulatum*

suggested that this plant could be used in the treatment of uncomplicated superficial infections caused by clinically important staphylococci, potentially pathogenic *S. aureus* or opportunistic *S. epidermidis* [15].

More interestingly, the antimicrobial activity of emodin isolated from *Rheum palmatum* was examined against 15 clinical isolates of MRSA. The results showed the MICs of emodine against *S. aureus* ranged from 1.56–25 μg/mL. This compoundvmarkedly lowered the MICs of amoxicillin and oxacillin tested against the same MRSA strains, resulting in a synergetic effect between this marker compound and these antibiotics [16].

Anthraquinones were detected in ethanol extracts along with acetone and ethyl acetate fractions of both fruits fibers and fruits cover of *Adansonia digitata* (baobab tree). Fruit covers extract exerted highest activity on tested bacterial agents (*B. subtilis, P. vulgaris, S. aureus*). In comparison to fruits fibers extract, fruits cover extract was more effective against fungi (*Aspergillus niger*), while both extracts did not show activity against *C. albicans* [17].

A bio-guided fractionation study was performed to evaluate the antibacterial activity of *Senna podocarpa* root hydro-ethanol extract against nine *Neisseria gonorrhoeae* reference and clinical strains, some with diminished susceptibility to penicillin, tetracycline, and ciprofloxacin. The results showed the anti-*N. gonorrhoeae* activity against all tested strains, with a MIC ranging from 100 to 400 μg/mL. Rhein, emodin, chrysophanol and physcione were isolated as main compounds and rhein (MIC = 3.13 μg/mL against all test strains) proved to be the most active of the isolates [18].

Emodin isolated from several *Cassia* species showed activity against *B. subtilis* (MIC = 7.8 μg/mL) and *S. aureus* (MIC = 3.9 μg/mL) but was inactive against two Gram-negative bacteria (*K. pneumoniae* and *E. coli*) at the highest concentration (500 μg/mL) tested [19]. It showed also weak activity against *S. pyogenes* and *S. typhi* (MIC = 3000 μg/mL) as well as *N. gonorrhoea* and *C. albicans* MIC = 4×10^3 μg/mL [20]. In another study it was indicated that, the antimicrobial effect of emodin against MRSA strains was higher than many antibiotics including imipenem, cefepime [21] or chloramphenicol [22]. Antimicrobial resistance can be defined as more than a fourfold increase in original MIC of antibiotic. In this case, there was no MIC increase for emodin during 20 passages, which suggested that MRSA did not develop resistance for emodin. For norvancomycin, MIC got a twofold increase at passage 4 and a fourfold increase at passage 15 which demonstrated the emergence of resistance. These results suggested it was not easy for emodin to cause MRSA resistance [21].

Antimicrobial activity of several anthraquinone derivatives such as 1,8-dihydroxy-2-[(z)-4-methylpenta-1,3-dien-1-yl] anthraquinone, 2-acetyl-3,8-dihydroxy-6-methoxyanthraquinone, emodin and glucofrangulin A, isolated from the methanolic extract of *Rhamnus cathartica* showed that 1,8-dihydroxy-2-[(z)-4-methylpenta-1,3-dien-1-yl] anthraquinone and emodin exhibited activity against *E. coli* and *S. aureus* and anti-yeast activity against *C. albicans*. The 2-acetyl-3,8-dihydroxy-6-methoxyanthraquinone only exhibited activity against *E. coli*. All compounds together with the methanol extract showed negative effect against *A. niger* [23]. On the other hand aloe-emodin was capable of inhibiting the growth of both Gram-positive and Gram-negative bacteria as well as inhibiting the growth of a nystatin resistant strain of the fungus *A. niger* [24].

Antimicrobial results obtained from the isolated compounds of *Tabebuia impetiginosa* dried inner bark showed that 2-(hydroxymethyl)anthraquinone exhibited strong activity against *H. pylori* ATCC43504 at 0.01 mg/disc. Anthraquinone-2-carboxylicacid and metronidazole were less effective, exhibiting moderate anti-*Helicobacter pylori* activity at 0.1 mg/disc. Amoxicillin and tetracycline were the most potent compounds tested, displaying very strong activity at 0.005 mg/disc. 2- (hydroxymethyl) anthraquinone exhibited moderate activity at this dose. Tetracycline still had strong activity at 0.001 mg/disc while amoxicillin had little activity at this dose [25].

The 8-methoxychrysophanol and other isolated compounds from *Asphodelus microcarpus* exhibited moderate antifungal activity against *C. neoformans* with an IC_{50} value of 15.0 μg/mL, while emodin, 10-(chrysophanol-7′-yl)-10-hydroxychrysophanol-9-anthrone and aestivin showed good to potent activity against MRSA with IC_{50} values of 6.6, 9.4 μg/mL and1.4 μg/mL respectively. Emodin and ramosin displayed good activity against *S. aureus* with IC_{50} values of 3.2 and 8.5 μg/mL [26].

The soranjidiol, rubiadin, damnacanthal and 5,5'-bisoranjidiol isolated from *Heterophyllaea pustulata* showed antibacterial activity against *S. aureus* with MICs between 32 μg/mL to 64 μg/mL [27].

An hexanic crude extract from leaves and roots of *Ceratotheca triloba* and its anthraquinones derivatives were also studied. Isolated compounds, 9,10-anthracenedione and 1-hydroxy-4-methylanthraquinone showed antibacterial activity against *S. aureus*, *Micrococcus luteus*, *B. cereus* and *Escherichia coli*. The crude extract exhibited good activity against *S. aureus* and *M. luteus*, medium activity against *E. coli* and *S. typhimurium* and very low activity against *B. cereus*. Although a similar trend was observed for 9,10 anthracenedione and 1-hydroxy-4-methyl anthraquinone, unlike the crude extract, a very low activity against *S. aureus* was observed for 9,10 anthracenedione and a high activity for 1-hydroxy-4-methylanthraquinone. Thus 9,10 anthracenedione is an effective drug against *E. coli* and *S. typhimurium* and 1-hydroxy-4-methylanthraquinone is effective against *S. aureus* and *M. luteus* [28].

The methyl-1,4,5-trihydroxy-7-methyl-9,10-dioxo-9,10-dihydroanthracene-2-carboxylate, also isolated from *Asphodelus microcarpus* showed a potent activity against MRSA and *S. aureus* with IC_{50} values of 1.5 and 1.2 μg/mL respectively [29]. Compounds identified as asphodosides B, C and D showed activity against MRSA with IC_{50} values of 1.62, 7.0 and 9.0 μg/mL, respectively. They also exhibited activity against *S. aureus* (non-MRSA) with IC_{50} values of 1.0, 3.4 and 2.2 μg/mL, respectively [30].

3. Reported mechanisms of actions of anthraquinones

Several mechanisms of actions of anthraquinones (as pure compounds or as marker compounds of crude extracts) were reported from different studies. In some cases, the indicated mechanisms were different from those of most common antimicrobial agents as it will be stated later in this section. As already stated the chemical structures of the main antimicrobial anthraquinones of this study are shown in Fig.1.

3.1 Action-mode studies

3.1.1 Cell wall synthesis and membrane function inhibition

Emodin, dose-dependently protected mice challenged with lethal dose of MRSA and decreased bacterial load in mice challenged with sublethal dose of MRSA. Morphology observation showed emodin might disrupt cell wall and membrane of MRSA. Although emodin had no influence on genes related to cell wall synthesis and lysis as well as β-lactamase activity and drug accumulation, it reduced membrane fluidity and disrupted membrane integrity [21].

Extracts of *Curtisia dentata* demonstrated high antimicrobial activity and low minimum inhibitory concentrations against *E. coli* (MIC, 100–2500 μg/mL), *Acinetobacter haemolyticus* (MIC, 100–850 μg/mL) and *Acinetobacter lwoffii* (MIC 150–2500 μg/mL), by inducing the leakage of Na^+ and K^+ from the tested bacteria as well as an inhibitory action against the expression of both Vtx1 and Vtx2 genes, responsible for the production of verocitotoxins [7].

3.1.2 Nucleic acid synthesis inhibition

In one study it was concluded that one of the antibacterial mechanisms of emodin was its ability to bind with the phosphate group of DNA and intercalate into the base pairs of the DNA helix. In this way it would affect replication and transcription, repress expression and even lead to cell death [14].

It has been demonstrated that anthraquinones extracted from different species of *Aloe* exhibit antibacterial activity by inhibition of nucleic acids synthesis in *B.subtilis* [31].

3.1.3 Other metabolic processes inhibition

Antimicrobial activity of 1,8- dihydroxyanthraquinones (DAD) is related to their inhibition activity on the enzymes which are necessary to microorganisms. Penicillase is inhibited by rhein, emodin and aloe-emodin. HIV-1 reverse-transcriptase is inhibited by hypericin. The activity of *N*-acetyltransferase in *H. pylori* decreases with increasing levels of rhein and the 1,8- DAD also exhibit antibacterial activity by inhibiting nucleic acid synthesis [10].

Emodin has shown a general antimicrobial effect against several microorganisms because of its capacity to interfere with cellular metabolism. It can cause inhibition of electron flow in the respiratory chain, most likely in between ubiquinone and cytochrome b, and also cause dissipation of the proton motive force [32], which may explain in part the anti-staphylococcal activity of *Rheum palmatum* extract. In one study, emodin showed minimal antibacterial activity against some Gram-negative MDR strains including *E. coli*, and *K. pneumoniae*, however, there was substantial enhancement of its activity with PaβN (efflux pump inhibitor) [22].

It was showed that anthraquinone-rich extracts, obtained from the phototoxic *Heterophyllaea pustulata* (Rubiaceae), exhibited bacteriostatic activity against *M. luteus* ATCC 9341, selectively inhibiting both oxacillin-sensitive and resistant *S. aureus*, and antifungal activity against important opportunist microorganisms and against those involved in superficial mycosis, all from nosocomial origin. In this context, soranjidiol, rubiadin, damnacanthal and (S)-5,5'-bisoranjidiol, showed *in vitro* bacteriostatic/bactericide activity against *S. aureus*. The mechanism of action seems to involve an increase in the levels of superoxide anion and/or singlet oxygen molecular.

The 5,5'-bisoranjidiol showed higher antibacterial activity than rubiadin and soranjidiol, although the three increase $O_2^{\cdot -}$ production at about the same level as a function of regardless that they are acting in darkness or under irradiation. In contrast, the $^1O_2$ production for 5,5'-bisoranjidiol was high irrespective of conditions (darkness or irradiation). This would suggest that, among the different species that comprise ROS (radical oxygen species), $^1O_2$ is the one mainly involved in the bactericidal effect. This increase in ROS could result from the interaction between bacteria and the anthraquinones without needing light [33], although, actinic irradiation generated a photosensitization for rubiadin, soranjidiol and 5,5'-bisoranjidiol, which consequently increased their antibacterial effects (particularly, bactericide) [27]. It's noteworthy that the bactericidal effect is suppressed when a specific quencher of $^1O_2$ is added, thus suggesting that the bactericidal activity derives mainly from that particular ROS [33].

Anthaquinones do not have simply antibactericidal effect but they are also able to decrease the pathogenicity of certain bacteria. Alizarin at 10 μg/mL was found to efficiently inhibit biofilm formation by three *S. aureus* strains and a *S. epidermidis* strain. Binding of Ca^{2+} by alizarin decreased *S. aureus* biofilm formation and a calcium specific chelating agent suppressed the effect of calcium. In addition, two other anthraquinones, purpurin and quinalizarin, were found to have antibiofilm activity. These three anthraquinones also markedly inhibited the hemolytic activity of *S. aureus*, and in-line with their antibiofilm activities, increased cell aggregation. Transcriptional analyses showed that alizarin

repressed the *α*-hemolysin *hla* gene, biofilm-related genes (*psmα*, *rbf*, and *spa*), and modulated the expressions of *cid*/*lrg* genes (the holin/antiholin system). These findings suggest anthraquinones, especially alizarin, are potentially useful for controlling biofilm formation and the virulence of *S. aureus* [34].

Some studies revealed that rhein has the ability to attenuate the virulence of *Porphyromonas gingivalis* by significantly reducing the expression of genes coding for important virulent factors, which are involved in bacterial adhesion, host defence mechanisms, tissue destruction, and nutrient acquisition [35], also rhein differentially regulated the expression of genes involved in bacterial physiology and pathogenicity of *S. aureus* [36-37]. These studies revealed the great potential for synergetic antibacterial activity between rhein and other class of antibiotics which might help to solve the problem of antibacterial resistance, once that pathogens are not exposed to these phytochemical compounds and therefore unlikely to develop resistances [35].

The combination effect of emodin with amoxicillin and oxacillin was found to be synergistic or partially synergistic. It was found that emodin reduced MICs of amoxicillin, tetracycline and oxacillin [16, 38] and showed antibacterial activity against *H. pylori* MDR possibly via mechanisms associated with altering *hefA* gene expression associated with efflux pump mechanism [38].

3.2 Structural-functional studies

In general, the anti-bacterial effects of emodin, rhein, and aloe-emodin are generally higher than those of physcion and chrysophanol. These anthraquinone derivatives have the same hydroxyanthraquinone nucleus composed of two ketone groups at C9 and C10 and two hydroxyl groups at C1 and C8, while different groups are substituted at C3 and C6 of the phenyl ring. Three anthraquinones (rhein, emodin, and aloe-emodin) have polar substituents - carboxyl, hydroxyl, and hydroxymethyl groups at C3, C6, and C3, respectively. It was reported that the presence of these polar functional groups could increase antibacterial activity. Although physcion and chrysophanol also have hydroxyl groups at C1 and C8, the apolar methyl and weakly polar methoxyl groups in chrysophanol and physcion might weaken their antibacterial activity [14].

In one study, anthraquinones isolated from *Vismia laurentii* were tested against a panel including Gram positive (*B. cereus*, *Listeria monocytogenes* and *S. aureus*) and Gram negative microorganisms (*E. coli*, *C. albicans* and *Salmonella enteritidis*). According to the obtained results, 3- geranyloxyemodin was bactericidal to the three Gram positive bacteria strains with activity increasing with pH while 3-methoxyemodin was active only on *S. aureus* with activity decreasing with pH. On the other hand 2-isoprenyl-3-methoxyemodin was active only at pH7 and only on *S. aureus* and *B. cereus* [9].

The antimicrobial activity of 1,8- dihydroxyanthraquinones (DAD) against some strains of bacteria depends upon their chemical structures. Rhein, emodin and 1,8-dihydroxyanthraquinone in decreasing order, inhibit the growth of *S. aureus*. However, the anti-bacterial activity of oxidized 1,8-DAD decreased when they changed to their reduced forms. The 1,8 -DAD are phenolic compounds contain hydroxyl groups in various positions of the anthraquinone molecule, so that they exhibit a various degree of antimicrobial activity, depending on the OH^- group [10].

In another study it was found that presence of an hydroxyl group in place of a methyl group at C3 or a methyl in place of hydroxyl group at C8 and an additional methyl ester (COOMe) group at C7 as in 3,6,8-trihydroxy-1-methylanthraquinone-2-carboxylic acid substantially reduced antimicrobial activities especially against the MRSA phenotype [22]. One more study that also stressed the importance of hydroxyl group position was the work on *Morinda angustifolia* root extract, which resulted in the isolation of 1,8-dihydroxy-2-methyl-3,7-dimethoxyanthraquinone (1), lucidin 3-*O*-*β*-primeveroside (2), 1,3-dihydroxy-2-methylanthraquinone (3), lucidin-ω-ethyl ether (4), lucidin-ω-butyl ether (5) and damnacanthol (6). Compound 1 (1,8-dihydroxy-2-methyl-3,7-dimethoxyanthraquinone) demonstrated significant higher antimicrobial activity against *B. subtilis*, *E. coli*, *M. luteus*, *Sarcina lutea*, *C. albicans* and *Saccharomyces* sp. in comparing to the others tested. Interestingly, only this compound possesses an additional hydroxyl group at C-8, which might suggest that the structural fragment with a carbonyl and two *β*-hydroxyls at a linear position in anthraquinones might be an important pharmacophore for the antimicrobial bioactivities [39].

A study of chemical structure-activity relationship study revealed that two hydroxyl units at the C-1 and C-2 positions of alizarin play important roles in antibiofilm and anti-hemolytic activities [34]. Isolated anthraquinones from roots dried powder of *Vismia laurentii* were classified in terms of decreasing antimicrobial activity such as 3-geranyloxyemodin (A), 3-ethoxyemodin (C), 2-isoprenyl-3-methoxyemodin (D), vismiaquinone (B) and bisvismiaquinone (E). It was assumed that steric effect, weight and the presence of substitutions in position 2 of emodin derivatives is detrimental to their bactericidal activity while increase in the aliphatic chain length of the methoxy substitution in position 6 is beneficial to the antibacterial activity of these emodin derived anthraquinones. Gram positive bacteria and *C. albicans* cells have their cell walls exposed, and compounds that can interact with these cell walls should have a long aliphatic chain to help disorder the cell wall. This was case of compound A compared to compounds C and D. Substitution in position 2 of the emodin was detrimental for the antibacterial activity of these compounds while the unsaturation of the substitute was noticed to be important for this activity. The increase of the aliphatic chain length of the methoxy substitute in position 3 increased the lipophilicity of the compound. The antimicrobial activity, which is increased by the lipophilicity of the compound, is reduced as the compound molecular weight increases [9].

4. Overall conclusion

Aloe-emodin, chrysophanol, emodin, physcion and rhein are the anthraquinones identified in nature with the highest number of studies proving their *in vitro* antimicrobial activity, against a set of microorganisms sensitive and resistant to antimicrobial drugs currently available for clinical use.

In general, extracts and isolated anthaquinones were active against Gram negative bacteria, particularly against *Pseudomonas aeruginosa, Helicobacter pylori and Neisseria gonorrhoeae* and Gram positive bacteria such as *Staphylococcus aureus*, namely MRSA strains and *S. epidermitis*. The synergic activity of anthraquinones with other antibiotics, resulting in a smaller MIC was also proved.

The antibacterial mechanisms of anthraquinones are diverse, including the simple destabilization of cell wall, alterations of metabolic pathways or DNA inclusions, in a direct or indirect way (via oxidative stress). The efficacy of these mechanisms is related to the molecular properties of the anthraquinone (steric effect, pH, polarity of group substituents). Additionally, the same anthraquinone derivative can have multiple mechanisms of action which makes it difficult for bacteria to develop resistances.

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