Antimicrobial and Antiviral Activity of Hydrolysable Tannins

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Abstract: Hydrolysable tannins (HTs), secondary metabolites widely distributed in the plant kingdom, are generally multiple esters of gallic acid with glucose. HTs have been shown to be effective antagonists against viruses, bacteria and eukaryotic microorganisms. The present review examines the antimicrobial and antiviral activity of HTs, the mechanism(s) of action, and some structure-activity relationships.

Key Words: Hydrolysable tannins, ellagitannins, gallotannins, tannic acid, antiviral activity, antibacterial activity, antimycotic activity.

INTRODUCTION

The worldwide search for new active compounds useful together with, or in substitution of currently used antimicrobial molecules has oriented the attention of academy and industry towards the development of novel drugs [1, 2]. This is particularly important in consideration of the increasing number of microbial genotypes that are resistant to many of the currently employed antimicrobial drugs. This problem, first described in bacteria, is now a significant threat also for the control of eukaryotic microorganisms. While many factors can be responsible for mutations in microbial genomes, it has been widely demonstrated that the incorrect use of antibiotics can greatly increase the development of resistant genotypes [1-6].

The antimicrobial properties of a number of secondary metabolites occurring in plant tissues have been known since antiquity [2, 7-9]. Among these, hydrolysable tannins (HTs) are secondary metabolites belonging to the family of "vegetable tannins" which have been broadly studied for more than 200 years. According to the Haslam-Bate-White definition, all vegetable tannins are characterized by being water soluble, and by having a molecular weight (MW) between 500 and 5,000 Da (or higher). They usually give a phenol reaction and express special properties such as the ability to precipitate alkaloids and protein [10]. An important group of HTs includes esters of gallic acid with glucose, the products of their oxidation and polymerization, and other oxidized and polymerized derivatives. From a chemical viewpoint, HTs are different from condensed tannins (*syn.* proanthocyanidins), which are derivatives of catechins, and tara tannins, which are esters of gallic acid with quinic acid [1-4].

Although the antimicrobial properties of HTs are well known, up to now most studies have not critically evaluated this activity. The present review will strive to present an overview of the current "state of the art" of the antiviral, antibacterial and antimycotic activity of HTs. Mechanism(s) of action and structure-activity relationships are also reviewed.

BIOSYNTHESIS OF HYDROLYZABLE TANNINS

In association with their biosynthetic pathway, HTs are classified into four types (I-IV). 1-Galloyl-β-D-glucose (syn. glucogallin) is presumed to be the first intermediate and a key metabolite in the synthesis of HTs. A specific glucosyl tranferase catalyzes the esterification between gallic acid and UDP-glucose to give glucogallin (Fig. **1**). In a second step, glucogallin plays a dual role, functioning as both as an acyl acceptor and donor, to give di- and trigalloyl-glucoses. This first biosynthetic pathway is completed by the formation of 1,2,3,4,6-pentagalloyl-β-D-glucose (generally considered to be a simple galloyl-glucose ester). During the second step the galloylation of pentagalloyl-glucose continues with the formation of hexa-, hepta-, octa-, etc. -galloyl-glucose derivatives [10-12]. These compounds constitute HTs type I and are also labelled "gallotannins" (GTs). Due to the presence of an esteric-link between two galloy moieties (depsidic link) (Fig. **1**), GTs are also considered to be depsidic metabolites. Tannic acid (TA), commercially available as a standardized extract, is a mixture of GTs characterized by having a high degree of polymerization with an elevated number of galloyl moieties. The third phase of the pathway yields ellagitannins (ETs) (HTs types II-IV), which are typical secondary metabolites of many plant families. ETs are produced *via* GT oxidation, which forms hexahydroxydiphenoyl (HHDP) (type II - e.g. tellimagrandin II), dehydrohexahydroxydiphenoyl (DHHDP) (type III - e.g. geraniin) and transformed dehydroellagitannin (type IV – e.g. chebulagic acid) derivatives (Fig. **2**) [10, 12, 13].

ETs with a HHDP group (including a C-C coupling between galloyl moieties esterified with the glucose scaffold) are widespread in nature. The main biaryl C-C coupling patterns (HTs type II) includes the presence of galloyl groups esterified in the 2,3- and 4,6-positions of the glucopyranose core, although coupling across the 1,6-, 1,3-, 3,6-, and 2,4-positions are also known (Fig. **2**). Further reactions result in the formation of C-glucosidic (HTs type II+, e.g. vesca-

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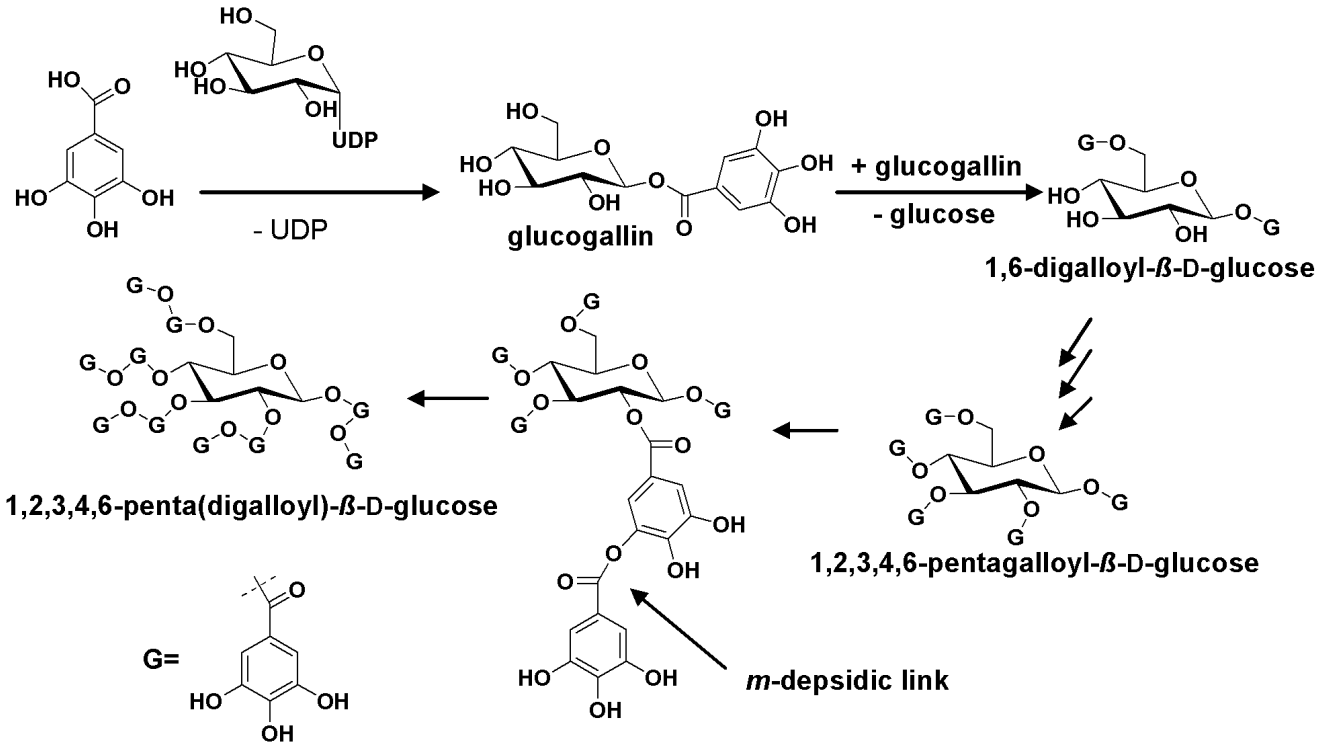


Fig. (1). Biosynthetic route of simple glucogalloyl derivatives and GTs with depsidic links (type I).

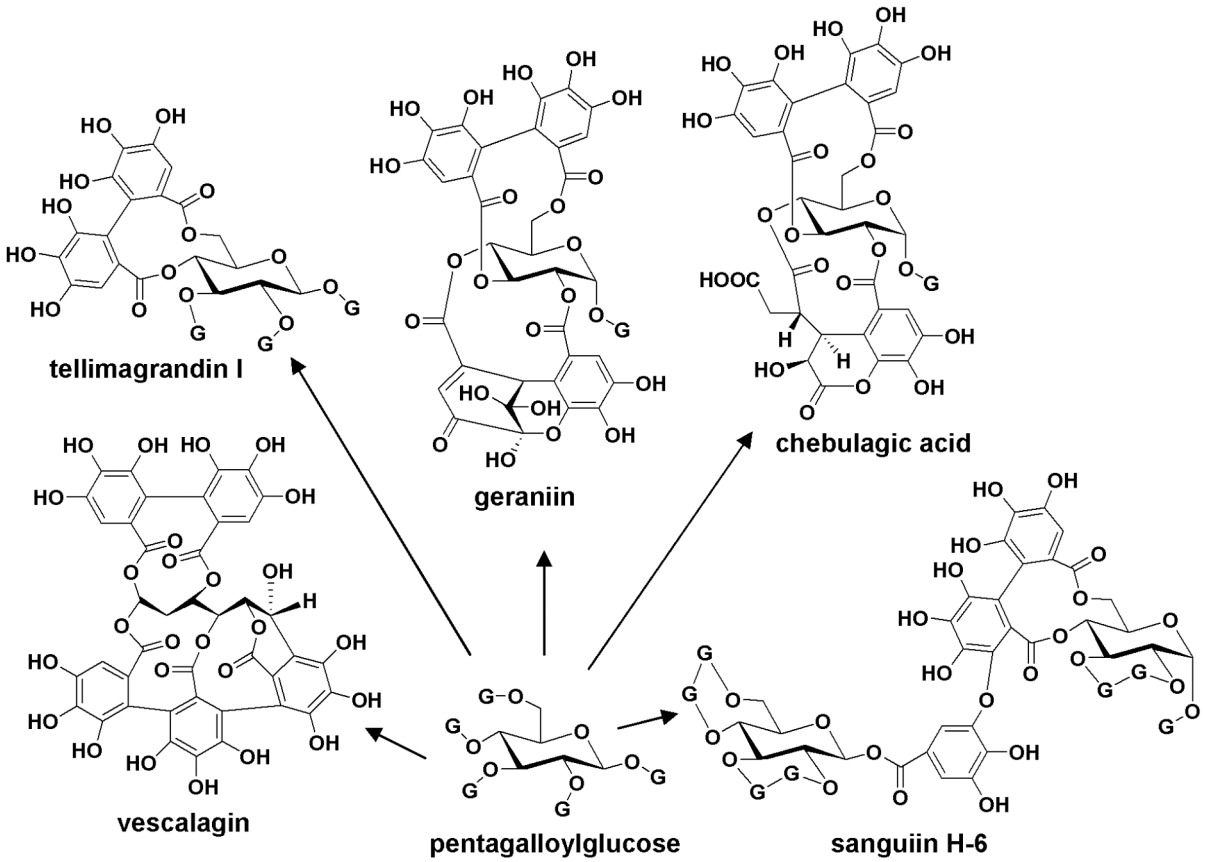


Fig. (2). Biosynthetic routes of ETs and 'complex' tannins (types II-IV).

lagin), oligomeric (e.g. sanguiin H-6) and polymeric ETs (obtained by the formation of C-C and/or C-O bonds between monomers) (Fig. **2**). The final step of the pathway is the oxidative formation of HTs type III (e.g. chebulagic acid) (Fig. **2**) and HTs type IV [10-16].

A number of excellent studies report the NMR structural characterizations of more than 1,000 HTs (from simple glucogallins with MW = 332 Da, to more complex polymers near 5,000 Da). These have been isolated from over twenty different plant families [10, 12, 14, 17-27].

In recent years new strategies for the study and development of antimicrobial compounds have been devoted to the synthesis of molecules similar to naturally occurring HTs. Even though the first synthesis of GTs was reported almost 100 years ago [11], only in the last fifteen years has it been possible to achieve the organic (total and model) synthesis of HTs (both GTs and ETs) [13, 16, 28-43]. In the last ten years some HTs characterized by structures similar or analogous to those found in nature have been synthesized. Accordingly, organic acids different from gallic acid (e.g. caffeic, benzoic and tri-methoxy gallic acid) or carbohydrates different than D-glucose (e.g. *O*-methyl-glucose, D-mannose, D-galactose, D-allose, D-xylose, D-arabinose, D-ribose and D-lyxose) have been used for the esterification reaction [34, 40, 42, 44-52].

ANTIVIRAL ACTIVITY OF HYDROLYSABLE TANNINS

Many HTs have been shown to display activity against some viruses at various stages of infection and replication. This has been particularly noteworthy towards *Herpes simplex* virus (HSV), human immunodeficiency virus (HIV), and leukemia virus [53- 63].

HSV is widespread in humans, particularly children or immuno-compromised patients [64]. After a primary infection, HSV tends to persist in the neuron of the ganglia and its reactivation may cause recurrent herpetic infection [65]. Early studies by Takechi *et al*. [66] and Fukuchi *et al*. [67] reported that both monomeric and dimeric HTs exhibited a remarkable *in vitro* anti-HSV activity. Cheng *et al*. [68] demonstrated that casuarinin (a HT isolated from *Terminalia arjuna*) is also characterized by a high anti-HSV activity, exhibiting a 50% inhibitory concentration (IC_{50}) ranging from 1.5 to 3.6 μM and virucidal activity at a concentration of 25 μM. Since the 50% cytotoxic concentration (CC_{50}) was quite high (about 89 μM), the selectivity index (SI = CC_{50} / IC_{50} ratio) was also elevated ranging from 25 to 59. Due to these high values, it is apparent that the *in vitro* anti-HSV activity of casuarinin is not due to its cytotoxicity [68].

Quideau *et al*. [63] studied five nonahydroxyterphenoyl-(NHTP) containing C-glycosidic ETs (castalagin, vescalagin, grandinin, roburin B, and roburin D, all found in *Quercus* spp. and *Castanea* spp.) (Fig. **3**). The authors tested the activity of these compounds towards cells infected by four HSV strains, two of which were resistant to Acyclovir (ACV), the most effective drug available against HSV-mediated diseases. ACV belongs to the family of nucleoside analog-type molecules and acts as a false building block for viral DNA synthesis [69]. Interestingly, all five of the ETs studied by Quideau *et al*. [63] displayed significant anti-HSV activities against both ACV-susceptible and ACV-resistant strains, which were from 5- to 50-fold higher than those exhibited by ACV. The most effective ET was vescalagin, which showed an IC_{50} of 0.04 nM [63].

Additonal studies of the activity of HTs towards other viruses include one focusing upon punicacortein C (a C-glycosidic ET) which exhibited an IC_{50} of 5 μM against the reverse transcriptase (RT) of human immunodeficiency virus (HIV) [53, 63]. Other investigations have shown that HTs can significantly inhibit both the cytopathic effect of human HIV and the expression of HIV antigen in human lymphotropic virus type I (HTLV-1)-pos MT-4 cells. The IC_{50} of the active compounds were 13- to 15-fold lower than their CC_{50} [57]. Finally, Ogata and Gato [70] found that the ET pedunculagin was active against RT in mouse-leukemia-virus-infected cells, with an IC_{50} of 128 nM.

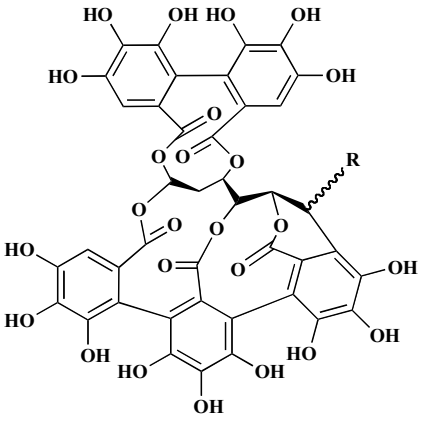
A study by Fukuchi *et al*. [67] on the mode of anti-HSV action of HTs hypothesized that TA inhibits virus attachment to cells. This was later confirmed by Cheng *et al*. [68] who demonstrated that casuarinin disturbs cell attachment and penetration, and also blocks the entrance of viral DNA into the nucleus during the late stages of cell infection. This is apparently achieved as casuarinin interferes with the fusion between the viral envelope and the plasma membrane by binding essential viral glycoproteins D, B, H and L [71, 72, 73] in a dose-dependent manner. Cheng *et al*. [68] also noted an 85% inhibition of viral penetration into the cell proper as early as 10 min after casuarinin addition [68].

An additional study by Quideau *et al*. [63] found that the C-glycosidic ETs castalagin, vescalagin, grandinin, roburin B, and roburin D (Fig. **3**) successfully inhibited HSV replication in cultured cells. Vescalagin in particular was shown to be extremely toxic and selective against ACV-resistant viruses with IC_{50} values about 10^7 times below that of ACV. Moreover, the corresponding SI values were approximately 10^5 times higher than that of ACV [63].

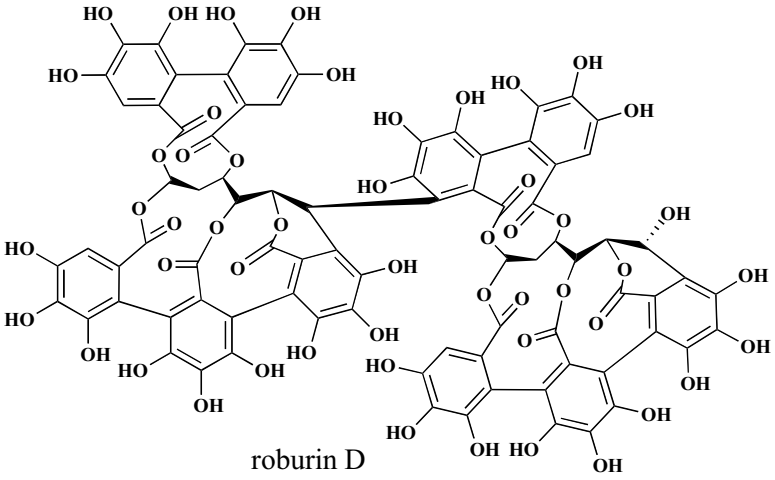
The mode of anti-HIV activity of HTs has also been studied. Free and biphenyl pyrogallol-type patterns have been shown to be useful pharmacophores for the inhibition of HIV-RT [56, 74, 75]. Nakashima *et al*. [57], on the other hand, demonstrated that the anti-HIV activity of HTs is mediated, at least in part, by the inhibition of HIV adsorption into the cell. Some possible mechanisms of HTs antiviral activity are summarized in Table **1**.

ANTIBACTERIAL ACTIVITY OF HYDROLYSABLE TANNINS

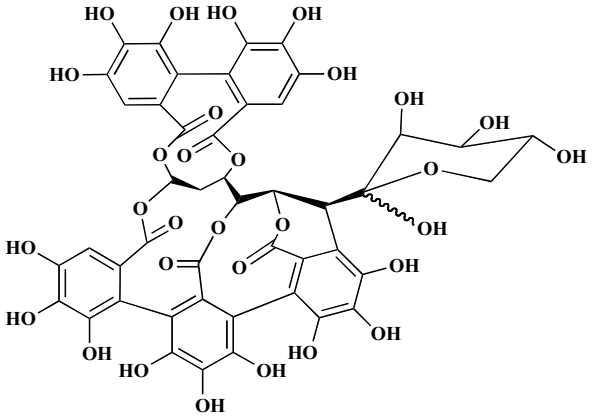
The antibacterial activity of HTs has also been extensively studied. Four HTs (punicalin α and punicalin β, punicalagin α and punicalagin β, isolated from *Punica granatum*) [76] have been shown to impede the growth of both methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) strains of *Staphylococcus aureus*. Purified forms of these HTs exhibited a minimal inhibitory concentration (MIC) of 62.5 μg/mL [77]. These results were in agreement with an earlier investigation of Burapadaja and Bunchoo [78] who



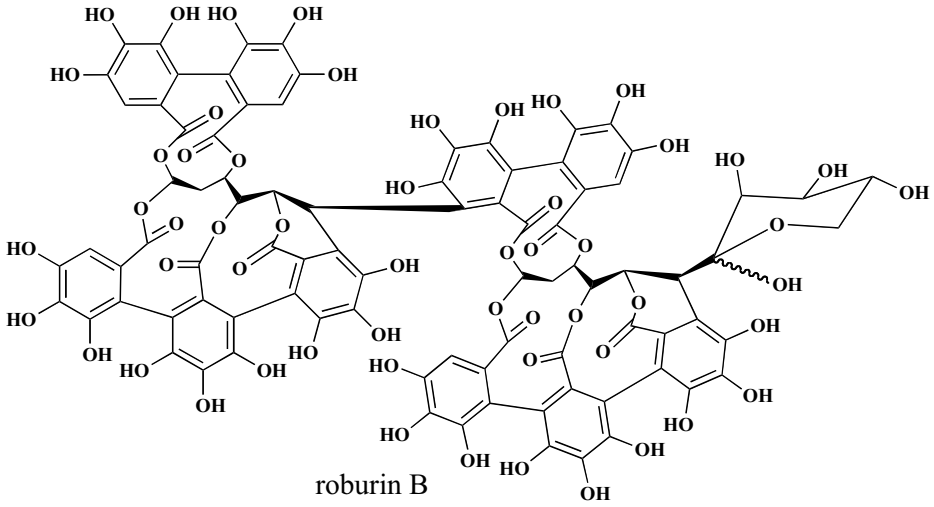
castalagin: R = α-OH
vescalagin: R = β-OH



roburin D



grandinin



roburin B

Fig. (3). Structures of castalagin, vescalagin, roburin (B and D), and grandinin.

successfully used punicalagin isolated from *Terminalia citrina*, although the use of unidentified bacteria as target microorganisms determined a difficult interpretation of data. More recently, an analogous inhibition towards MRSA was observed using the HTs tellimagrandin I and corilagin [79-81]. Another investigation by Akiyama *et al*. [82] examining the activity of TA on plasma coagulation by *S. aureus* showed that after incubation for 24 h bacterial activities were inhibited by 100 μg/mL.

Table 1. Mechanism of Antiviral Action Hypothesized for Some HTs

Compound	Viral Target	Mechanism of Action	References
Tannic acid	*Herpes simplex* virus (HSV)	inhibition of virus adsorption to the cells	[67]
Hydrolysable tannins	human immunodeficiency virus (HIV)	inhibition of virus adsorption to the cells	[57]
Hydrolysable tannins	*Herpes simplex* virus (HSV)	inhibition of virus adsorption/penetration to the cells; inhibition of the virus penetration into cell nucleus	[68]
C-Glycosidic ellagitannins	human immunodeficiency virus (HIV)	inhibition of virus reverse transcriptases	[63]

High cell surface hydrophobicity (CSH) appears to be directly related to the virulence of bacteria associated with gastrointestinal infections [83]. Annuk *et al*. [84] found that aqueous extracts from bearberry and cowberry leaves decreased the CSH of *Helicobacter pylori*. Although a rigorous analytical characterization of the active components was not performed, the authors speculated that TA could be active against both CSH and *H. pylori* activity. On the contrary, a study by Funatogawa *et al*. [85] suggested that HTs might act directly on bacterial surface structures. During an investigation of 20 purified HTs against *H. pilori* they showed that monomeric HTs are active. Particularly interesting was the ability of tellimagrandin I to rapidly provoke the aggregation of *H. pilori* cells.

Several screening surveys have been carried out on the antibacterial activity of HTs towards large sets of target bacterial species. TA has been shown to inhibit the growth of *Bacillus anthracis*, *Pseudomonas aeruginosa Salmonella* spp., *Shigella dysenteriae*, *S. aureus*, and *Streptococcus pneumoniae* in a dose-dependent manner [86]. Likewise, de Miranda *et al*. [87] found that TA has bactericidal activity against salivary bacteria. Other studies [88, 89] investigating the antimicrobial activity of bark extracts of *Combretum molle* and *Rhizophora apiculata* against Gram-positive and Gram-negative bacteria hypothesized that the observed activity could be due to the presence of HTs. Although some interesting results have been reported, the lack of an analytical characterization of crude extracts makes the results scarcely exploitable for pharmaceutical applications.

The presence of HTs in extracts of *Melaphis chinensis* has been known since the 1960's. GTs, which represent the major constituents (>90%), have been reported to contain a penta-*O*-galloylglucose nucleus to which three or four additional galloyl groups are attached [90]. These compounds exhibited bactericidal effects against *Actinomyces viscosus, Streptococcus mutans* and *Streptococcus salivarius*. They were also shown to inhibit *in vitro* adherence to glass, glucosyltransferase (GTF) activity, glucan-induced agglutination, and growth of various strains of *S. mutans* [91].

Studies of the mode of HTs antibacterial activity have been carried since the 1980s. However, since most lack a rigorous analytical characterization of the active compounds, many conclusions still need to be confirmed. One of these: the suggestion that HTs interact with proline-rich proteins occurring in the salivary pellicle or on cell-surface lipoteichoic acid [92, 93], was confirmed by Wu-Yuan *et al*. [91] who found that GTs present in ethanolic extracts of *M. chinensis* strongly inhibited *in vitro* GTF adherence and glucan-induced agglutination of *S. mutans* and *Streptococcus sobrinus*. Since the ability of GTs to inhibit plaque formation is directly related to an ability to alter the initial attachment of *S. mutans* (resulting in reduced bacterial adherence and cariogenesis), the authors postulated that their observations suggested the presence of HT-protein interactions. Wolinsky and Sote [93], on the other hand, observed that HTs from *Serindeia warnecki* could partially suppress glucan synthesis.

In recent years, an interaction between ETs and proteins of high molecular weight has been hypothesized. Machado *et al*. [77] suggested that after being adsorbed ETs react with the protein moiety of cell enzymes (e.g. oxidoreductases) occurring both in the cytoplasm and in the cell wall while another study proposed an impediment of bacterial adhesion as a result of a modification of cell surface receptors [2]. Finally, Funatogawa *et al*. [85] concluded that the observed

Table 2. Mechanism of Antibacterial and Antimycotic Action Hypothesized for Some HTs

Compound	Microbial Target	Mechanism of Action	References
Gallotannins	*Streptococcus* spp.	interaction with proline-rich proteins or cell-surface lipoteichoic acid	[92, 93]
Gallotannins	*Streptococcus* spp.	Inhibition of glucosyltransferase	[91]
Ellagitannins	*Staphylococcus aureus*	interaction with cytoplasm and wall enzymes (e.g. oxidoreductases)	[77]
Hydrolysable tannins	*Helicobacter pylori*	damaging activity of lipid bilayer membranes	[85]
Hydrolysable tannins	*Candida albicans*	damaging activity of cell wall and membrane	[89]

antibacterial activity of HTs was the consequence of the dose-dependent damaging activity towards lipid bilayer membranes, although the effective relationship between cell membrane damage and antibacterial activity were not fully elucidated. Some mechanisms of HTs antibacterial activity are summarized in Table **2**.

ANTIMYCOTIC ACTIVITY OF HYDROLYSABLE TANNINS

A series of HTs (glucogallin, corilagin, pelargoniin, and phyllantusiin) isolated from *Pelargonium reniforme* were evaluated for their antimycotic activity against opportunistic yeasts and filamentous fungi. While none of the compounds stopped fungal growth, some activity was noted towards some yeast species such as *Candida albicans* with MICs from 16 to 125 μg/mL [94]. Additional studies [88, 89] reported that HTs derived from the bark of *Rhizophora apiculata* showed significant antimycotic activity towards opportunistic yeasts. Nevertheless, the absence of an analytical characterization of the extracts makes the interpretation of those results problematic. On the other hand, a partial confirmation of those results could be from another study showing that TA can inhibit the growth of some species of the genera *Candida*, *Cryptococcus*, *Filobasidiella*, *Issatchenkia*, and *Saccharomyces* [95].

Until now the mechanism of the antimycotic activity of HTs has not been widely studied. A single study by Lim *et al*. [89] postulated a damaging effect of cell wall of *C. albicans*. Membrane observations using scanning (SEM) and transmission (TEM) electronic microscopy showed significant morphological changes (especially at the level of cell wall and membrane) after exposure to HTs (Table **1**). Also in this case, since accurate analytical characterization of extracts was missing, these hypotheses will have to be confirmed by a more rigorous investigation. In spite of these analytical shortcomings, in the light of the interesting results of many studies, a recent patent has been formulated regarding the use of HTs derived from plant extracts as antimycotic drugs against yeasts and yeast-like microorganisms [96].

SYNERGY BETWEEN HYDROLYSABLE TANNINS AND ANTIBIOTICS

Recent studies have shown that co-treatments employing antibiotics together with HTs result in significant synergistic effects allowing for a reduction in the amount of antimicrobials used. Early studies found that corilagin (isolated from *Arctostaphylos uva-ursi*) and tellimagrandin I (from *Rosa canina*) markedly reduced the MICs of β-lactam antibiotics (e.g. oxacillin and cefmetazole) against MRSA strains [80, 81]. Corilagin was more effective than tellimagrandin I (reduction by 100- to 2,000-fold of the MIC for oxacillin against MRSA). An explanation for this synergistic effect was recently provided by Shiota *et al*. [97] who found that both HTs significantly reduce the production of the penicillin binding protein 2'(2a) [PBP2'(PBP2a)] which confers resistance to β-lactam antibiotics by MRSA. They also were able to limit the activity of β-lactamases. The high specificity of these interactions was confirmed by the absence of synergy between corilagin and other antimicrobial agents such as benzylpenicillin, erythromycin, fosfomysin, ofloxacin, streptomycin, tetracicline and vancomycin [81]. Akiyama *et al*. [82] also found that TA increased the activity of β-lactam drugs (e.g. oxacillin) towards *S. aureus*, although no mechanistic hypothesis was proposed.

More recently some synthetic glucogalloyl esters were evaluated for their antimycotic activity in association with polyene antibiotics. When a mixture of α- and β-anomers of *O*-methylgluco-2,3-digalloyl esters was used in association with amphotericin B, a marked reduction of the MIC for this polyene against a panel of *Candida* spp. strains was observed [44]. The authors hypothesized that the presence of two aromatic rings (each containing three hydroxyl groups) in *O*-methylgluco-2,3-digalloyl esters could possibly protect the polyene structure of amphotericin B against oxidation [44].

STRUCTURE-ACTIVITY RELATIONSHIP STUDIES OF HYDROLYSABLE TANNINS

Due to the scarcity of studies using purified molecules, only a few investigations have strived to explain the structure-activity relationships (SAR) of HTs. Quideau *et al*. [63] compared the anti-HSV activity of castalagin, vescalagin, grandinin, roburin B, and roburin D (Fig. **3**), all characterized by having HHDP units, as well as the overall number of OH groups. The presence of HHDP- and NHTP- units in these compounds was recognized as a key feature for specific interactions with viral targets such as protein and/or polynucleotide helical complexes [43, 98, 99]. Of the above mentioned ETs, vescalagin was shown to be the best inhibitor of virus replication, while its epimer castalagin was surprisingly less active against both ACV-susceptible and ACV-resistant HSV strains. In spite of their relatively high molecular weights (934 Da), both diastereoisomeric compounds have rather compact structural architectures while exhibiting significant configurational differences. In agreement with Vivas *et al*. [100], molecular-modeling studies indicated that castalagin is slightly more stable than vescalagin [63]. As first postulated by Yoshida *et al*. [101], this could be due to the β-oriented C(1)-OH group of vescalagin, which is directed outward from the less-crowded face of the molecule, whereas the same group in castalagin is oriented in such as way as to be available for a stabilizing H-bond between the O-atom and the H-atom of the phenolic 4'-OH group of the galloyl ring I in the NHTP unit. The formation of this intramolecular H-bond in castalagin, but not in vescalagin, could explain the different reactivity of the two structures. Quideau *et al*. [63] hypothesized that vescalagin exhibits a greater anti-HSV activity respect to castalagin since this configurational difference evidently consents a greater ability to recognize HSV molecules. The same author also observed that the larger compounds ETs roburin B and roburin D are less active and less selective respect to castalagin and vescalagin. On the other hand, lyxose-containing grandinin exhibited effective and selective inhibition of HSV replication in ACV-resistant, but not in ACV-susceptible strains, thus suggesting a predetermined virus specificity. Finally, a number of studies found that some monomeric, dimeric and oligomeric structures exhibited similar anti-HIV activity. Some of these include: the monomer gemin D [102], the dimers nobotanin B [103] and camelliin B [104], and the tetramer trapanin B [19, 57, 60].

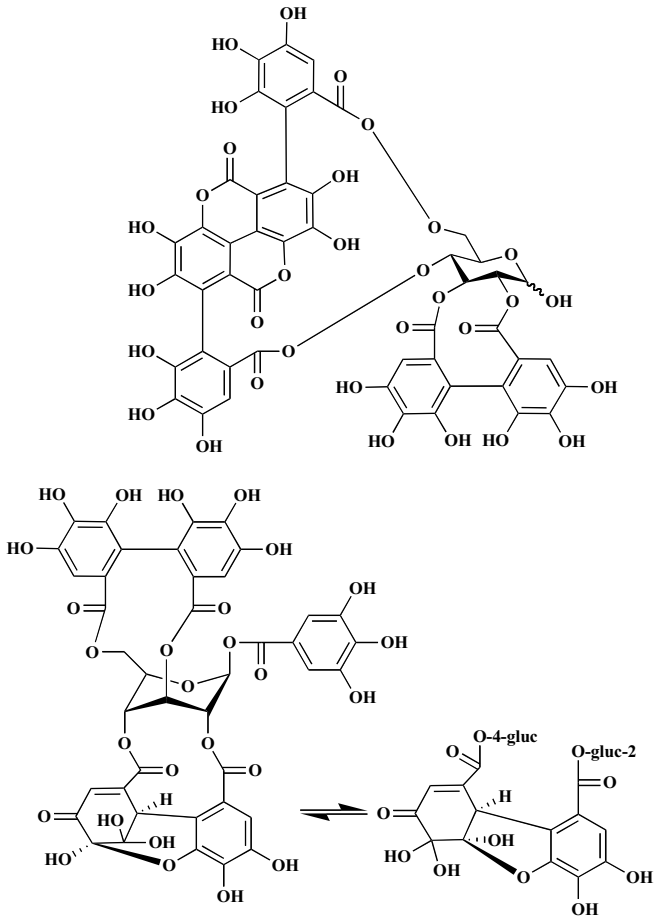


Fig. (4). Structures of punicalagin and geraniin.

SAR studies on the antibacterial activity of four different HTs: TA, castalagin (Fig. **3**), punicalagin, and geraniin (Fig. **4**) against food-borne pathogenic bacteria (*S. aureus*, *Salmonella* spp., enteropathogenic strains of *Escherichia coli* (EPEC), and *Vibrio* spp.) were carried out by Taguri *et al*. [105]. All four HTs exhibited similar MICs against *S. aureus*. However, activities against the other three bacterial groups were less significant and appeared to be structure dependent. All four HTs are characterized by having 3,4,5-trihydroxybenzoyl structures (Figs. **3** and **4**). In particular, punicalagin is an ester of a HHDP and gallagyl group, whereas TA is a mixture of polygalloyl glucopyranose. Surprisingly, despite having significant structural differences, with the sole exception of the anti-*E. coli* activity, these two HTs showed similar antibacterial properties. Geraniin (characterized by an oxidized HHDP group) exhibited low activities against species of the genus *Salmonella* and *E. coli*. Finally, castalagin showed the strongest activity against *E. coli, Salmonella* spp. and *S. aureus*.

Similar to the results obtained towards bacteria, the antimycotic activity of HTs against yeasts has been ascribed to the presence of HHDP moieties in these molecules. In addition, it appears that the presence of pyrogallol elements in some structures is crucial for their antimycotic activity against *Candida* species. On the contrary, neither number of hydroxyl groups nor molecular size seem to be deterninant for antimycotic activity [94, 106].

CONCLUDING REMARKS

As reported by Scalbert [107], it is likely that tannin activity depends upon the variability of individual target microorganisms. This could explain the differences observed among bacteria, yeasts or filamentous fungi.

Current literature reported in this review underlines the fact that HTs comprise a class of compounds extensively studied as potential antiviral and antimicrobial drugs. Although some promising results have been obtained, most studies have been confined to the laboratory and for various reasons no HT has so far been up-graded for the commercial exploitation as an antimicrobial drug.

The lack of rigorous analytical determinations of HTs occurring in phenol fractions is prevalent in most of the literature cited in this review. Studies using purified (or partially purified) molecules are still very few in number. This is probably due to the scarcity of appropriate protocols for HT analysis. The improvement of analytical techniques will undoubtedly lead to a better knowledge of the antimicrobial properties of individual HTs. The lack of commercially available pure HT standards represents an additional problem, and synthetic strategies, which are costly and time-consuming, can only represent a partial solution to this problem.

The assay protocols employed for the *in vitro* evaluation of HTs antimicrobial activity is another problem. In most studies activities was checked by only using preliminary testing methods (e.g. disk diffusion assay on agar dishes, or similar procedures). Moreover, most of the studies reporting MICs did not apply standard CLSI guidelines [108, 109], including the use of quality control (QC) strains, essential for monitoring and confronting the accuracy of the method. As a result, the low reproducibility of results obtained in studies not employing standard CLSI guidelines makes the data relatively useless for pharmaceutical applications.

The situation could be considerably improved if particular attention were directed towards the use of reference (antibiotic) compounds. In addition, the activity of each HT against a given pathogenic microorganism (and its cytotoxic potential) should always be referred to one or more reference compounds. If missing, results are virtually useless according to present standards. Unfortunately, this consideration can be applied to most of the previously published studies.

Another problem undermining most HT studies is the well-documented strain-related susceptibility of microbial genotypes. As a result, the use of undetermined (or even unidentified) strains should be discouraged. Certified strains coming from recognized microbial culture collections should be preferred. In addition, large sets of target (reference) strains should be routinely used. Only by respecting standard protocols will it be possible to compare and confront results obtained in different laboratories.

It is also important to point out that until now HT activity investigations have been conducted using *in vitro* studies in which direct effects against a given pathogenic microorganism were presented. Even though useful as a preliminary investigation, *in vivo* activity is clearly more significant. Many questions remain open, in fact, regarding the ability of

HTs to reach the microbial cell target in the human body. In addition, even if these compounds can reach the target microorganism, would they maintain the same conformations? To the authors' knowledge, although essential for the possible exploitation of HTs for therapeutic purposes, no answer to these fundamental questions has been so far provided by current literature.

A multidisciplinary approach aimed at evaluating the effectiveness of different HT structures as antiviral and antimicrobial drugs should be emphasized and encouraged. This is particularly important considering the high percentage of microbial resistance to currently used antibiotics, and in view of the well documented synergistic effects obtainable by combining HTs with routinely used antibiotics.

ABBREVIATIONS

HTs = Hydrolysable tannins

GTs = Gallotannins

ETs = Ellagitannins

TA = Tannic acid

MW = Molecular weight

HHDP = Hexahydroxydiphenoyl

DHHDP = Dehydrohexahydroxydiphenoyl

NHTP = Nonahydroxyterphenoyl

DCC = Dicyclohexylcarbodiimide

DMAP = 4-dimethyl-aminopyrinide

IC_{50} = 50% inhibitory concentration

CC_{50} = 50% cytotoxic concentration

SI = Selectivity index

HSV = *Herpes simplex* virus

HIV = Human immunodeficiency virus

ACV = Acyclovir

MIC = Minimal inhibitory concentration

MRSA = Methicillin-resistant *Staphylococcus aureus*

MSSA = Methicillin-sensitive *Staphylococcus aureus*

CSH = Cell surface hydrophobicity

GTF = Glucosyltransferase

SEM = Scansion electronic microscopy

TEM = Transmission electronic microscopy

PBP2' = Penicillin binding protein 2'(2a) (PBP2a)

SAR = Structure-activity relationship

EPEC = Enteropathogenic strains of *Escherichia coli*

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