# Evaluation of the Antimicrobial Potency of Tannins and Related Compounds Using the Microdilution Broth Method

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***Abstract:*** **The antimicrobial activity of a total of 27 tannins and related compounds was evaluated against 8 microorganisms, including 2 Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*), 4 Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*), and 2 yeasts (*Candida albicans*, *Cryptococcus neoformans*). The compounds tested were generally found to possess only weak to moderate antibacterial, but fairly high anticryptococcal activities. Attention is given to structure-activity relationships with emphasis on simple galloyl esters, hydrolyzable tannins and proanthocyanidins among this class of secondary products.**

Tannins and related compounds have long been recognized to possess quite potent antibiotic activities, reflected by recorded uses of traditional herbal medicines rich in polyphenols as effective antiseptic drugs (1–3). In a recent authoritative review, tannin toxicity for fungi, bacteria and yeasts is summarized and some reasonable mechanisms are presented to explain tannin antimicrobial activity (4). However, most of the previous antimicrobial studies have only been carried out with crude tannin fractions. Therefore, in the present study, the antimicrobial potential of a series of chemically defined tannins and related compounds was evaluated to ascertain whether polyphenols tend to be highly potent or just very moderate antimicrobial agents.

Although the series of tannins and related compounds tested for their antimicrobial potencies in this study does not reflect the amazing structural diversity of this group of natural products, each sample represents a chemically defined compound of high purity. The antimicrobial spectrum and the minimum inhibitory concentrations (MICs) of the samples are displayed in Table **1**. As can be seen, gallic acid methyl ester (**2**) shows higher antibacterial activity relative to its parent phenol, gallic acid (**1**), an observation which is consistent with previous reports (5). This finding may be attributed to the difference in polarity, facilitating the permeation through the cell wall. Comparison of the activity of shikimic acid (**3**) (data not shown) with its 3-*O*-galloyl (**4**) and 3,5-di-*O*-galloyl analogs (**5**) indicated some dependency on the degree of galloylation. However, perusal of the MIC values of all tested galloyl esters clearly showed that both the number of galloyl groups and the molecular size, two structural features that have been repeatedly found to be major contributing factors toward pharmacological activities, are not significant regarding antimicrobial potency. The ellagitannins corilagin (**8**) and phyllantusiin C (**9**) were generally found to possess only weak antibacterial activities with MICs ranging from 1000–2000 μg/ml. In addition, the present data suggest greater susceptibility for yeasts when compared to bacteria.

Although **1** itself displayed a remarkable antifungal activity against *Cryptococcus neoformans* indicated by an MIC value of 250 μg/ml, introduction of additional galloyl groups does not necessarily enhance this particular biological activity as reflected by **5** and **7**. Similarly, enhancement in potency cannot be correlated to the number of hydroxy group in these active compounds. Here, the presence of a hexahydroxydiphenoyl moiety or its oxidatively modified entities may be an important structural feature leading to highly potent anticryptococcal compounds such as **8** and **9**.

The picture that emerged from examining the antimicrobial potencies of the proanthocyanidins – clearly indicated only weak to moderate activities with most of the MIC values between 1000–2000 μg/ml, irrespective of the configuration of both relative 2,3-*cis* and 2,3-*trans* stereochemistry of constituent units and orientation of the interflavanyl linkage. It also appeared that an increase in molecular weights, i.e., dimers **12–17** and **21–27** vs. the trimer **16** and hexamer **17**, at least at these levels, did not enhance antimicrobial potency. For the oligomer **17**, also the presence of galloyl groups at C-3 of flavan-3-ol entities did not confer tannin toxicity. Comparison of the phloroglucinolic B-type proanthocyanidins (**12–17**) with their 5-deoxy analogs (**21–27**) showed similar moderate antibacterial activities for both groups of proanthocyanidins against the panel of bacteria. While the B-types (**12–17**) and the 5-deoxy analogs (**21–27**) reflect some fundamental hydroxylation structural variants on the A-ring, the non-availability of prodelphinidins precluded structure-activity relationships regarding B-ring substitution patterns under the experimental conditions employed. Previous studies showed that the number of hydroxy groups on the B-ring affect the level of growth inhibition of certain microorganisms (4).

The negligible antimicrobial effects, observed for the doubly-linked A-types (**18–20**), indicated that molecular rigidity imposed by the introduction of an additional ether linkage between the two flavanyl units does not enhance the antimicrobial properties.

It appears from this study that the potential of chemically defined polyphenols to inhibit the growth of microorganisms is less prominent than commonly anticipated, at least within the range of compounds tested and for these microbial species. Nevertheless, polyphenols do have the potential for affecting microorganisms. A plausible explanation of moderate antimicrobial activities of distinct polyphenols, but effective antimicrobial protection to plants and the claimed efficacy of traditional herbal medicines rich in polyphenols anti-infectious agents may be concluded from both the presence in plants and herbal preparations in large concentrations and the chemical heterogeneity of the pro polyphenols which could well counterbalance the

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**Table 1** Antimicrobial activity of simple galloyl esters, hydrolyzable tannins, flavan-3-ols and proanthocyanidins (broth microdilution method; MIC values in μg/ml).

| Compound | *E. coli* | *Klebs. pneum.* | *Bac. subtilis* | *Staph. aureus* | *Pseud. aerug.* | *Prot. mirab.* | *Cand. albic.* | *Crypt. neofor.* |
|---|---|---|---|---|---|---|---|---|
| *Galloyl Esters* | | | | | | | | |
| **1** gallic acid | 2000 | 1000 | 500 | 500 | 500 | 500 | 1000 | 250 |
| **2** gallic acid Me ester | 500 | 250 | 500 | 250 | 250 | 250 | 1000 | 500 |
| **4** 3-*O*-galloyl-shikimic acid | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **5** 3,5-di-*O*-galloyl-shikimic acid | 500 | 500 | 500 | 500 | 1000 | 1000 | 500 | 500 |
| **6** glucogallin | 500 | 1000 | 500 | 250 | 1000 | 1000 | 500 | 500 |
| *Hydrolyzable Tannins* | | | | | | | | |
| **7** hamamelitannin | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 | >2000 |
| **8** corilagin | 1000 | 1000 | 2000 | 250 | 1000 | 1000 | 500 | 250 |
| **9** phyllantusiin C | 1000 | 2000 | 1000 | 1000 | 1000 | 1000 | 500 | 125 |
| *Flavan-3-ols* | | | | | | | | |
| **10** catechin | >8000 | >8000 | >8000 | >8000 | >8000 | >8000 | n.d. | n.d. |
| **11** 3-*O*-galloyl-catechin | 1000 | 1000 | 1000 | 2000 | 2000 | >2000 | 1000 | 1000 |
| *B-Types* | | | | | | | | |
| **12** | 1000 | 1000 | 1000 | 1000 | 1000 | 2000 | 500 | 250 |
| **13** | 2000 | 2000 | 1000 | 1000 | 1000 | >2000 | 1000 | 500 |
| **14** | 2000 | 2000 | 1000 | 1000 | 500 | 1000 | 1000 | 500 |
| **15** | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 500 |
| **16** | 1000 | 2000 | 1000 | 1000 | 1000 | 1000 | 1000 | 500 |
| **17** | 2000 | 1000 | 1000 | 1000 | 2000 | 1000 | 500 | 500 |
| *A-Types* | | | | | | | | |
| **18** | 1000 | 2000 | 2000 | 500 | 1000 | 2000 | 2000 | 2000 |
| **19** | 1000 | 2000 | 2000 | 1000 | 1000 | 2000 | 2000 | 2000 |
| **20** | 1000 | 2000 | 2000 | 1000 | 1000 | 2000 | 2000 | 2000 |
| *5-Deoxy Analogs* | | | | | | | | |
| **21** | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| **22** | 1000 | 1000 | 1000 | 1000 | 1000 | 2000 | 1000 | 1000 |
| **23** | 1000 | 1000 | 2000 | 1000 | 1000 | 2000 | 1000 | 1000 |
| **24** | 1000 | 1000 | 1000 | 1000 | 1000 | 2000 | 1000 | 1000 |
| **25** | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| **26** | 1000 | 2000 | 1000 | 2000 | 500 | 2000 | 1000 | 500 |
| **27** | 1000 | 2000 | 1000 | 2000 | 1000 | 2000 | 1000 | 1000 |
| *Reference Agents* | | | | | | | | |
| penicillin G | 125 | 125 | 125 | 125 | 62 | 62 | – | – |
| nystatin | – | – | – | – | – | – | 16 | 16 |

n.d. = not determined.

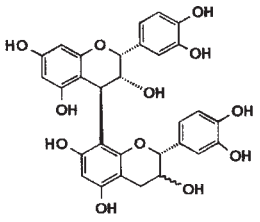
toxicity for microorganisms. The indicated parameters, polyphenol concentration and composition, thus define, at least in part, the quality of polyphenolic herbal medicines and provide for potential therapeutic benefits in the non-specific medical treatment of infectious conditions such as diarrhoea and skin diseases. The general picture that is now beginning to emerge from our recent study is that anti-infectious protection in a human body may be attributed to both direct bactericidal effects, whenever possible, and stimulation of the non-specific immune system (6). This does not exclude possible specific pharmacological effects of distinct polyphenols in diseased states.

## Materials and Methods

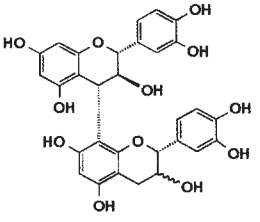
Tannins examined in this report were available as reference samples in our research group, shikimic acid (**3**), flavan-3-ols (**10**, **11**), procyanidin dimers (**12**–**15**), and catechin-(4α,8)-catechin-(4α,8)-catechin (**16**), or isolated: gallic acid (**1**), its methyl ester (**2**), glucogallin (**6**), corilagin (**8**), and phyllantusiin C (**9**) from *Pelargonium* spp. (7); hamamelitannin (**7**) and oligomer (**17**) were from *Hamamelis virginiana* L. (8); the A-type compounds (**18**–**20**) were from *Aesculus hippocastanum* L. (9), and the 5-deoxy compounds (**21**–**27**) from *Pithecellobium dulce* (Roxb.) Benth. (10). The shikimic acid derivatives (**4** and **5**) were kindly provided by Dr. Nishioka, Japan.

The above mentioned compounds were tested against a panel of microorganisms including, the Gram-negative bacteria, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* V 6089, *Proteus mirabilis* ATCC 14153, *Pseudomonas aeruginosa* ATCC 27853, the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, and the yeasts, *Candida albicans* ATCC 10231 and *Cryptococcus neoformans* 70219, DSM, Braunschweig.

Minimum inhibitory concentrations (MICs) of the samples were determined for susceptible microorganisms by a standard twofold microdilution technique using Mueller Hinton Broth, with penicillin G (bacteria) and mystatin (fungi) as reference agents (11).



12: ⌇ = |
13: ⌇ = ⋮



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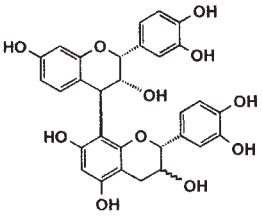

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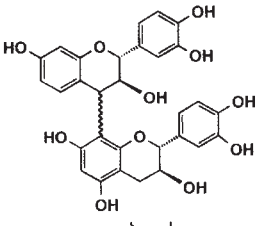
22 ⌇ = ⋮



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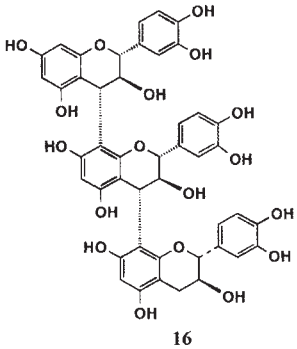

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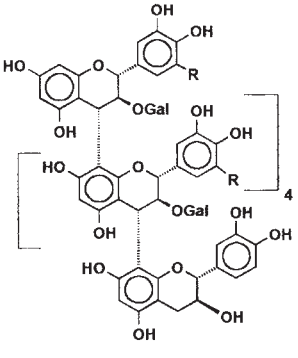
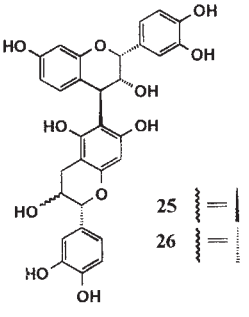
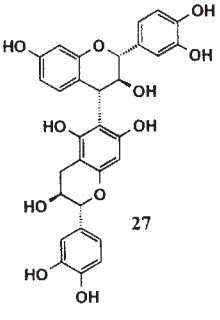


17 R = H or OH



25 ⌇ = |
26 ⌇ = ⋮



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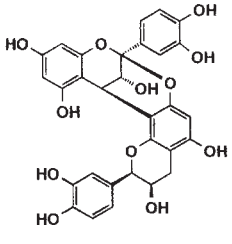

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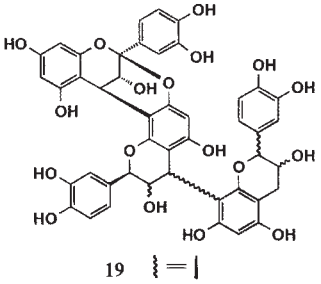

19 ⌇ = |
20 ⌇ = ⋮

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