



## NOTE

### Phytol May Inspire New Medicinal Foods for the Treatment of Heart Disease

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This study aimed to investigate *in vitro* antibacterial activity of phytol against the selected Gram positive bacteria (*Clostridium sporogenes*, *Enterococcus faecalis* and *Sarcina lutea*) involved in the pathogenesis of infective endocarditis. The examined natural product has proved to be active against all the tested bacteria, but to a varying degree. Indeed, phytol inhibited *E. faecalis* growth (MIC  $1.56 \pm 0.04$   $\mu\text{g/mL}$ ) more effectively than gentamycin and ampicillin (MIC  $5.00 \pm 0.06$  and  $16.00 \pm 0.03$   $\mu\text{g/mL}$ , respectively). Both its freely presence in nutrition and easy availability support the development of a new phytol based medicinal foods targeting heart disease.

**Keywords:** Diterpene alcohol, Antibacterial activity, *Enterococcus faecalis*, Infective endocarditis.

Traditional Chinese medicine suggests that some mosses of the genus *Rhodobryum* (Bryaceae) can be used as crude drugs for heart disease<sup>1</sup>. Actually, this genus includes four species present in northern hemisphere<sup>2</sup> together with some additional ones from subtropical or tropical regions<sup>3</sup>. The moss *Rhodobryum ontariense* has a wide but very fragmented distribution<sup>4</sup>. Preliminary analysis of its volatiles has indicated phytol as the main constituent (31.95 %)<sup>5</sup>. Such abundance of this natural product has not previously been reported in essential oils of other mosses<sup>6</sup>.

Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) is an acyclic monounsaturated diterpene alcohol, present in vitamin K, vitamin E and other tocopherols. It is an active ingredient in formulations that lower serum levels of triglycerides and/or cholesterol<sup>7</sup>. Indeed, this compound may be administered both to patients (*e.g.* with type II diabetes, obesity or heart disease) and healthy individuals<sup>8</sup>.

A large body of evidence implicates number of microbial strains in the pathogenesis of infective endocarditis (IE)<sup>9,10</sup>. Enterococci are the third most common etiologic agent of IE worldwide after *Staphylococci* and *Streptococci* being responsible for 10 to 15 % of cases<sup>11</sup>. Approximately 90 % of the enterococcal endocarditis cases are caused by *Enterococcus faecalis*, with < 5 % by *E. faecium*. The bacteria *Clostridium sporogenes* and *Sarcina lutea* are also listed among the microorganisms which might provoke IE<sup>12,13</sup>.

The aim of this study was to investigate *in vitro* antibacterial activity of phytol against three IE bacteria for the first time.

The examined chemical was used as received from Sigma-Aldrich, Munich, Germany (97 %, mixture of isomers), without any further purification. The Gram-positive bacteria *Clostridium sporogenes* ATCC 19404, *Enterococcus faecalis* ATCC 19433 and *Sarcina lutea* ATCC 9341 were obtained from the Mycological Laboratory, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia. The antibacterial assay was carried out by a 96 well microdilution method<sup>14</sup>. Gentamycin (Sigma-Aldrich, Munich, Germany) and ampicillin (Panfarma, Belgrade, Serbia) were used as a positive control, respectively. All experiments were performed in duplicate and repeated three times.

Phytol has showed to be active against all the tested bacteria, with MIC and MBC values 1.56-6.25 and > 12  $\mu\text{g/mL}$ , respectively (Table-1).

The most susceptible bacterium was *E. faecalis*. Actually, this organic compound inhibited *E. faecalis* growth more effectively (MIC < 2  $\mu\text{g/mL}$ ) than gentamycin and ampicillin (MIC 5 and 16  $\mu\text{g/mL}$ , respectively). The reports on phytol antibacterial activity are relatively scarce. Rajab *et al.*<sup>15</sup> reported a MIC value of 2  $\mu\text{g/mL}$  against the IE bacterium *Mycobacterium tuberculosis* for (*E*)-phytol, (*Z*)-phytol, commercially available 2:1 mixture of (*E*) and (*Z*)-phytol and (3*R*,5*R*,7*R*,11*R*)-

TABLE-1  
MINIMUM INHIBITORY (MIC) AND BACTERICIDAL (MBC) CONCENTRATIONS OF PHYTOL

| Bacteria                                 | Phytol <sup>*†</sup> | Gentamicin <sup>*‡</sup> | Ampicillin <sup>*‡</sup> |
|--|----------------------|--------------------------|--------------------------|
| <i>Clostridium sporogenes</i> ATCC 19404 | 3.12 ± 0.08 / >12    | 2.50 ± 0.02 / > 20       | 64.00 ± 0.06 / > 128     |
| <i>Enterococcus faecalis</i> ATCC 19433  | 1.56 ± 0.04 / > 12   | 5.00 ± 0.06 / > 20       | 16.00 ± 0.03 / > 128     |
| <i>Sarcina lutea</i> ATCC 9341           | 6.25 ± 0.06 / > 12   | 2.50 ± 0.03 / > 20       | 2.00 ± 0.03 / > 2        |

\*MIC/MBC (µg/mL); †Positive control; ‡p < 0.05

phytanol, respectively. Furthermore, the derivatives (*E*)-phytol acetate, a mixture of the (2*S*,3*S*)- and (2*R*,3*R*)-isomers of (*E*)-phytol epoxide and (3*R*,*S*,7*R*,11*R*)-phytanic acid displayed lower activities with MICs of 8, 16 and > 128 µg/mL, respectively<sup>15</sup>. On the other hand, Pejin *et al.*<sup>16</sup> reported a MIC value of 3 µg/mL against the IE bacterium *Listeria monocytogenes* for the phytol sample screened herein.

Phytol is a common and nonmutagenic food additive, with satisfactory safety. In addition, it is structurally simple, easily available and cost-effective chemical<sup>17</sup>. The low toxicity and high tolerance by mammals make this terpene molecule good candidate for the key ingredient of a new medicinal foods targeting heart disease.

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