New unnatural gallotannins: Synthesis and biological activity evaluation using various *in vitro* assays

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Introduction

The therapeutic potential of natural and synthetic antioxidants associated with oxidative damage is a source of inspiration for design of new biologically active structures. Reactive oxygen species (ROS) production can alter cell viability by disrupting DNA, proteins and lipids. Such oxidative damage leads to neurodegenerative, inflammatory or cardiovascular diseases and cancer. [1,2] Polyphenolic compounds act as a strong antiradical agents mainly due to their redox properties, which make them efficient hydrogen donors, reducing agents and metal chelators. Various naturally occurring polyphenols, including gallic acid, possesses a wide range of biological activities, of which a strong antioxidant activity is the most known. Polyphenolic compounds can be found free in nature or in a combination with sugars, generally as glycosylated derivatives – gallotannins. [3] The aim of this study was focused on investigation of the radical scavenging/antioxidant properties of structurally different synthetic gallotannins (GTs), alkyl gallates, and their influence on DNA damage. [4]

Material and methods

Several compounds bearing the gallate moiety, such as gallic acid (3,4,5-trihydroxybenzoic acid, **GA**), methyl gallate (**MG**) octyl gallate (**OG**) and gallotanins 2,3,4,6-tetra-O-galloyl-methyl-D-mannoside (**G_4Man**), 2,3,4-tri-O-galloyl-methyl-L-rhamnoside (**G_3Rham**) and 2,3,4,6-tetra-O-galloyl-methyl-D-glucoside (**G_4Glc**) were used for comparison of their properties. Potencial DNA protecive or genotoxic effect of the selected gallates and gallotanins was analyzed by the DPPH test, the reducing power assay (FRAP), the DNA topology assay and comet assay.

Antioxidant effect

The results from **DPPH assay** indicate that gallotannins **G_3Rham**, **G_4Man** and **G_4Glc** exhibited a higher radical scavenging effect in comparison with alkyl derivatives **MG**, **OG** and **GA** (**Fig.1**). In the **FRAP assay** they exhibited slightly higher and concentration-dependent reducing power compared to alkyl gallates and GA. (**Fig.2**).

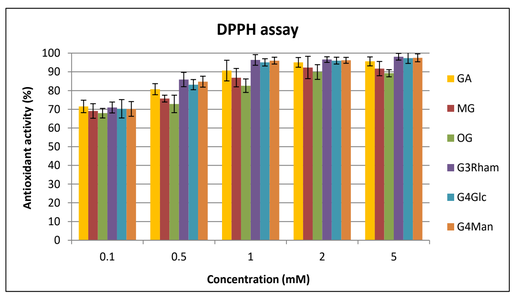

Fig. 1 DPPH radical scavenging assay - percentage of radical scavenging activity of MG, OG, G_3Rham, G_4Man and G_4Glc in different concentration compared with positive control GA.

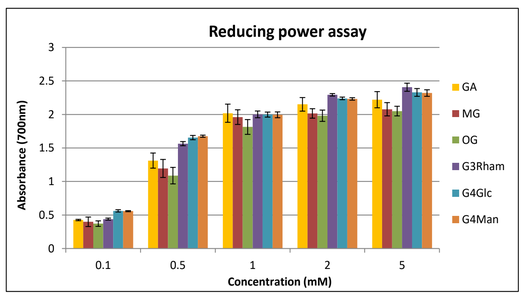

Fig. 2 Reducing power assay – reducing power of MG, OG, G_3Rham, G_4Glc, G_4Man in different concentration compared with positive control GA.

Genotoxicity

The results from **comet assay** indicate that gallotannins **G_3Rham**, **G_4Man** and **G_4Glc** exhibited significantly lower genotoxic effect in comparison with **MG**, **OG** and **GA** (**Fig.3A**).
The human blood lymphocytes pre-treated with the studied compounds, after the exposure to hydrogen peroxide (oxidant) were significantly protected by GTs, as seen from the **combined comet assay** (**Fig.3B**).

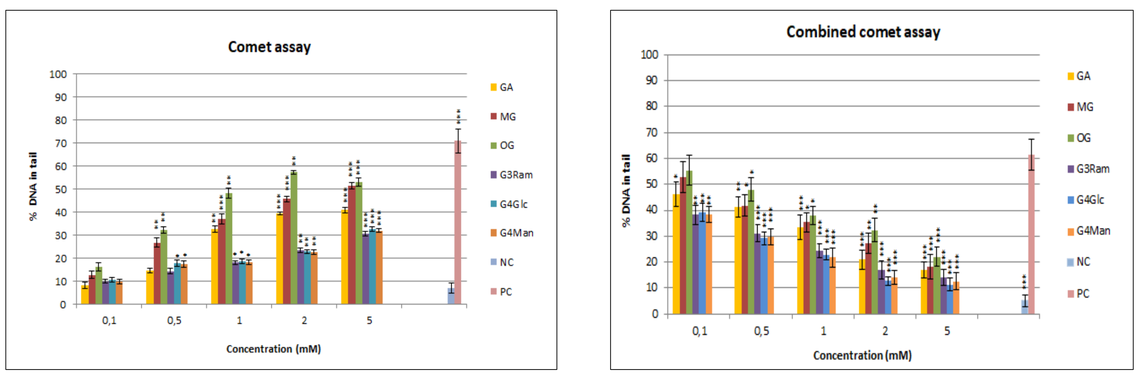

Fig. 3. Comet assay. DNA damage caused by treatment with studied compounds in human lymphocytes. G_4Glc, G_4Man, G_3Rham, MG, OG and GA. Cells treated with PBS - positive control (PC); cells treated with H_2O_2 -negative control (NC). (**A**) standard alkaline comet assay **(B)** combined comet assay, lymphocytes pre-treated with the studied compounds, after the exposure of hydrogen peroxide (400 μM H_2O_2, 5 min, 4ºC). Cell damage is expressed as the percentage of DNA in the comet tails. Data are reported as mean values ±SD of each compound tested in triplicate, *p<0,05; **p<0,01; ***p<0,001.

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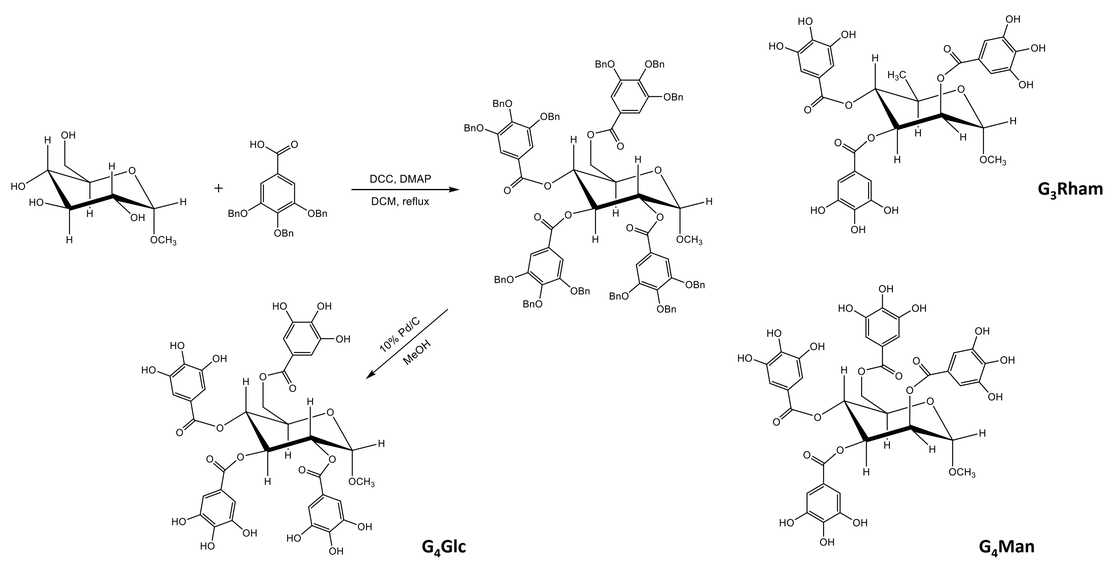
Synthesis

Synthetic gallotannins **G_4Man**, **G_4Glc** and **G_3Rham** were prepared by Steglich esterification method from unprotected methyl glycosides and benzylated gallic acid. Subsequent deprotection of OBn groups yielded studied GTs.



DNA-protective ability against oxidative damage

The promising activity of studied unnatural GTs to protect plasmid DNA against oxidant-induced DNA damage makes **G_3Rham**, **G_4Man** and **G_4Glc** valuable alternatives to currently used antioxidants. (**Fig.4**).

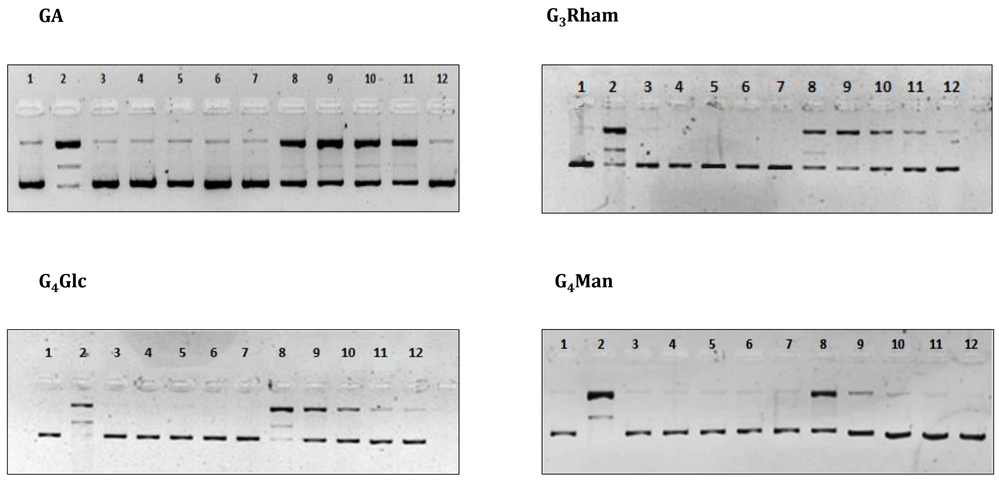


Fig. 4. DNA topology assay – GA and derivatives of GA (dGA) (gallotaninns) – G_3Ram, G_4Glc and G_4Man. The compounds were tested for their potential genotoxic effect in the presence of plasmid DNA (pDNA) (bands 3–7) and antigenotoxic effect in presence of Fe^{2+} ions (bands 8–12); **band 1**: negative control (pDNA); **band 2**: positive control (pDNA + Fe^{2+}); **band 3**: pDNA+ 0,1 mM dGA; **band 4**: pDNA+ 0,5 mM dGA; **band 5**: pDNA+ 1 mM dGA; **band 6**: pDNA+ 2 mM dGA; **band 7**: pDNA+ 5 mM dGA; **band 8**: pDNA + Fe^{2+} + 0,1 mM dGA; **band 9**: pDNA + Fe^{2+} + 0,5 mM dGA; **band 10**: pDNA + Fe^{2+} + 1 mM dGA; **band 11**: pDNA + Fe^{2+} + 2 mM dGA; **band 12**: pDNA + Fe^{2+} + 5 mM dGA.

Conclusions

The structure-biological activity relationship studies showed that alkyl gallates and synthetic gallotannins possess:

- a **strong antioxidant properties** as confirmed through the DPPH and the FRAP assay.
- the ability to **protect pDNA** against **oxidative DNA damage** was determined by the DNA topology assay.
- a good correlation was found between the antioxidant activity and the reducing power of tested compounds.
- combining **phenolic** and **carbohydrate moiety** should **improve** their **solubility**, **biocompatibility** and potential applications in cosmetic and pharmaceutical applications.

References

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