Screening of the antibacterial activity of phenolic extracts from Portuguese north-eastern plants

E. Pinho[1], L. Barros[2,3], G. Soares[4], I. C.F.R. Ferreira[2] and M. Henrriques[1]

[1] IBB–Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal
[2]CIMO/Escola Superior Agrária, Instituto Politécnico de Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal
[3] Grupo de Investigación en Polifenoles (GIP-USAL), Facultad de Farmacia, Universidad de Salamanca, Campus Miguel de Unamuno, 37007 Salamanca, Spain
[4] Department of Textile Engineering, University of Minho, Campus Azurém, 4800-058 Guimarães, Portugal

Thermal injury, trauma, chronic ulcerations, pressure or venous stasis result in lost of skin integrity allowing the deposition and colonisation of the injury tissue by a wide range of bacteria. Skin and soft tissues infection are typically colonized by staphylococci or streptococci, but virtually any microorganism may induce tissue inflammation and immune response. The severity of the infection may range from self-limit superficial infections to life-threatening diseases. However, the most common treatment for skin and soft tissue infections is antibiotics. However the indiscriminate use of this kind of drugs affects the normal skin flora and may result in multi-resistant strains. In order to overcome this issue it is critical to identify new antimicrobial agents.

Nowadays, the synthetic antimicrobial agents have been replaced by natural ones, since these are more friendlly to the user and are more available. Plants are, virtually, inexhaustible sources of biologically-active compounds, responsible for defence mechanisms against microorganisms, insects and herbivores. Therefore, bioactive compounds from plants have been widely used in the food, cosmetic and pharmaceutical industries. Such molecules include polyphenolics, alkaloids and polysaccharides, and they, all, have pharmacological properties, such as anti-inflammatory, antimicrobial and antioxidant properties.

Several ethonobotanical surveys conducted in the north-eastern region of Portugal by Ana M. Carvalho sellected some wild plants used on folk pharmacopeia and tradicional cusine as potential antimicrobials. In the present work, a screening of the antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, usually associated with skin infections, was performed using nine phenolic extracts of wild plants and eight coumpounds from selected extracts. The phenolic extracts were characterized by HPLC–DAD-ESI/MS. The antibacterial activity of the extracts and the compounds was assed by the disk difussion assay described on National Committee for Clinical Laboratory Standards (NCCLS), M27-A2 document (NCCLS, 2002), with some modifications and the minimal inhibition concentration (MIC) was obtained by the method described by Wiegand et al, 2008[a].

The results obtained from the disk diffusion assay allowed the selection of the phenolic extracts with higher activity against the three bacteria strains. The phenolic extracts from the *Cistus ladanifer, Castanea sativa, Filipendula ulmaria* and *Rosa micrantha* showed halo formation for all bacteria. Hence, the MIC of these four phenolic extracts was evaluated, the values ranged between 0.313 $mg.mL^{-1}$of extract (*Cistus ladanifer*/*K. pneumoniae*) and 2.5 $mg.mL^{-1}$ (*Filipendula ulmaria*/*S. epidermidis, K. pneumoniae; Rosa micrantha*/ *S. epidermidis, K. pneumoniae*). Antimicrobial activity of the most important compounds present on the selected extracts was measured by the disk diffusion method. Gallic acid was the compound with the highest activity for all bacteria. Thus, it was used in combination with other compounds in order to predict synergism/antagonism among them. The results showed that the activity of gallic acid was reduced or even annulled by the other compounds used. Moreover, ellagic acid, caffeic acid, gallic acid, kaempferol, rutin, quercetin and chrysin exhibited halo formation at least for one of the bacteria used, thus their MIC was assessed. Gallic acid was the one with the lowest MIC value (9.75, 39 and 19 $\mu g.mL^{-1}$ for *S.aureus*, *S. epidermidis* and *K. pneumoniae*, respectively), while ellagic acid had the highest values (>5 $mg.mL^{-1}$ for all bacteria strains).

Overall, the phenolic extract of *C. ladanifer*, *C. sativa, F. ulmaria* and *R. micrantha* inhibit the growth of the most common bacteria found on skin infections and gallic acid was the phenolic compound present in the extracts with higher activity.

Keywords Phenolic compounds; HPLC-DAD-ESI/MS; Wound infections; Antibacterial activity

[a] Wiegand et al. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances, Nature Protocols 3:2 163-175.