

Antioxidant properties of medicinal plants as potential antiatherosclerotic remedies: *in vitro* and *ex vivo* studies

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Oxidative and glycoxidative stress are postulated to be the primary events in the pathogenesis of type 2 diabetes mellitus and its vascular implications. Further, LDL oxidation in the vessel wall plays a key role in atherogenesis, also related to damage from oxygen species (ROS). Moreover, the risk for development of atherosclerosis is by approximately three-fold increased in patients with diabetes. The medicinal plants are widely used in folk medicine for the treatment of cardiovascular diseases and diabetes mellitus. The genus *Casimiroa* (Rutaceae) includes few species which have their habitat in Central America and Mexico; among these, the most common are *Casimiroa edulis* Llave et lex. and *Casimiroa pubescens* Ramirez. The decoction of leaves and seeds are traditionally used for treating hypertension. The sap of *Croton lechleri* (Euphorbiaceae), a South American tree, is used topically in the treatment of wound healing and orally, in a dilute form, mainly for gastric ulcers and intestinal diseases. The gum resin of *Boswellia serrata* (Burseraceae), which grows in dry mountainous regions of India, Northern Africa and Middle East, has been traditionally used to treat various chronic inflammatory diseases. *Ribes nigrum* (Grossulariaceae), a species native to central and northern Europe and northern Asia, is a traditional medicine for the treatment of inflammatory disorders such as rheumatic diseases.

The aim of this research was to investigate the antioxidant activity of these medicinal plants by means of several experimental methods *in vitro* and *ex vivo* to outline their role in the prevention and/or treatment of cardiovascular diseases related to oxidative stress.

The characterization of each plant extract was carried out by high performance liquid chromatography with diode array detection (HPLC-DAD). The antioxidant activity was evaluated by DPPH method [1], and ORAC (Oxygen Radical Absorbance Capacity) assay [2]. Also, the total phenolic content (TPC) [3] was determined by the use of Folin-Ciocalteu reagent, and the total flavonoid content (TFC) by complexation with chloride aluminium [4]. The activity of the plant extracts on LDL oxidation was studied by monitoring the formation of conjugated dienes [5], and the quantification of thiobarbituric acid reactive substances (TBARS) [6]. Finally, the advanced glycation end products (AGEs) were evaluated in human LDL by the use of periodate assay [7].

In DPPH assay, *Croton lechleri* sap and blackcurrant (*Ribes nigrum*) bud extract showed higher scavenging activity in comparison with *Casimiroa* extracts, whereas in the ORAC assay the *Casimiroa* leaf extracts showed a high ORAC value and *Croton lechleri* an activity even higher. In TPC test, *Croton lechleri* showed the highest value (552.5 ± 37.3 mg GAE/g). In the LDL oxidation assay, the plant extracts exhibited considerable protective effects by prolonging the oxidation lag phase; for example, at the concentration of 0.8 $\mu\text{g/ml}$ *Croton lechleri* increased the lagtime by 59%. *The results from this research suggest that the medicinal plants Croton lechleri, Casimiroa spp. and Ribes nigrum, even if in different manner, may have implications in the prevention of atherosclerotic vascular diseases, whereas Boswellia serrata showed a minor role.*

References

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