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Bioguided fractionation and isolation of natural inhibitors of advanced glycation end-products (AGEs) from *Calophyllum flavoramulum*

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ABSTRACT

Advanced glycation end-products (AGEs) are associated with many pathogenic disorders such as Alzheimer's disease, pathogenesis of diabetes, atherosclerosis or endothelial dysfunction leading to cardiovascular events. Clusiaceae and Calophyllaceae families are rich in compounds like polyphenols which are able to inhibit their formation and are therefore of great interest. *Calophyllum flavoramulum* Hend. & Wyatt-Sm., a native Malaysian plant, was selected after an anti-AGEs screening conducted on DCM and MeOH extracts from plants belonging to these aforementioned families. In a first study, bioguided fractionation of the MeOH leaf extract of *C. flavoramulum* afforded amentoflavone, 3-methoxy-2-hydroxyxanthone, 3,4-dihydroxy-tetrahydrofuran-3-carboxylic acid, quercitrin, 3,4-dihydroxybenzoic acid, canophyllol and apetalactone. Amentoflavone and 3-methoxy-2-hydroxyxanthone were found to be very potent (IC_{50} = 0.05 and 0.06 mM respectively), while anti-AGEs activities of quercitrin and 3,4-dihydroxybenzoic acid appeared as moderately strong (IC_{50} = 0.5 mM). In a second study, a systematic phytochemical study of the cyclohexane, DCM and EtOAc extracts obtained from the same plant was conducted to isolate the following products: flavoramulone, 6-deoxyjacareubin, rheediachromenoxanthone, 2,3-dihydroamentoflavone and benzoic acid. 3,4-Dihydroxy-tetrahydrofuran-3-carboxylic acid and flavoramulone were isolated for the first time and their structures were identified by means of IR, MS and NMR spectrometries.

1. Introduction

Advanced glycation end-products (AGEs) are involved in the pathogenesis of diabetes (Brownlee, 2001; Singh et al., 2001) and neurological diseases such as the Alzheimer's disease (Takeuchi and Yamagishi, 2008). AGEs contribute to the development of atherosclerosis (Jandeleit-Dahm and Cooper, 2008), joint diseases (DeGroot, 2004) and they are also responsible for aging and tissue damage (Grillo and Colombatto, 2008). As illustrated in Fig. 1, the interaction of AGEs with specific receptors (RAGEs) leads to an increase in production of reactive oxygen species (ROS) and activates the transcription factor NF-κB. In various types of cells, the transcription of NF-κB regulated genes induces an inflammation process (Alexiou et al., 2010; Brownlee, 2001; Srikanth et al., 2011) and pathological changes (Brownlee, 2001). Therefore, compounds able to break AGEs, or inhibit their formation, may be considered as potential drugs, dietary supplements or other bioactive ingredients (Khalifah et al., 1999; Monnier, 2003; Peng et al., 2011; Reddy and Beyaz, 2006). These considerations have prompted the scientific community to identify and develop new anti-AGEs compounds, acting either *via* trapping of reactive dicarbonyl species, preventing oxidation using transition metal chelators or free radical scavengers, or breaking AGEs cross-linking (Reddy and Beyaz, 2006). Among various synthetic compounds exhibiting anti-AGEs properties (Derbré et al., 2010), some of them were clinically evaluated towards cardiovascular diseases, such as diabetic nephropathy and retinopathy. Although some products have been discontinued (*e.g.* alagebrium chloride ALT-711, aminoguanidine), others are currently under investigation in phase II clinical trials (GLY-230, pyridoxamine dihydrochloride and TRC-4186) (Pharmaprojects, 2011).

Being the innermost layer of blood vessels cells, the vascular endothelium is in direct contact with the circulating blood. This tissue is now recognized as essential in the regulation of vascular tone and structure. In particular, it synthesizes and releases substances (*e.g.* nitric oxide NO) not only acting on the contraction and proliferation of smooth muscle cells, but also on inflammation,

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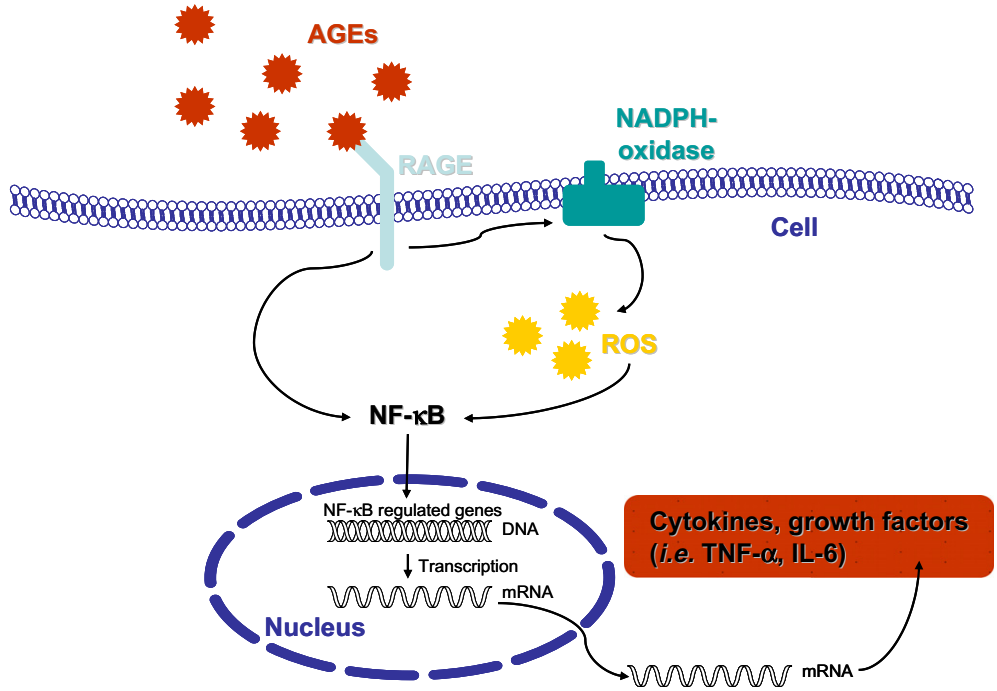

Fig. 1. Pro-inflammatory NF-κB signalling through AGEs receptors (RAGEs) Brownlee (2001) and Srikanth et al. (2011).

platelet aggregation as well as clotting. Under regular physiological conditions, the endothelium controls the release of these substances maintaining a balance between pro and anti-atherogenic effects. In case of endothelial dysfunction, this balance is broken and pro-atherogenic effects take over, increasing cardiovascular risks. As an endothelial dysfunction always precedes cardiovascular events, the identification of agents preventing the former could slow down the cardiovascular alteration and avoid clinical events (Versari et al., 2009). The implication of AGEs in endothelial dysfunction is now well-known (Brownlee, 2001; Versari et al., 2009), so the identification of anti-AGEs molecules addresses this issue. With this aim, we recently developed a rapid fluorescence-based anti-AGEs assay (Derbré et al., 2010). Indeed, while the anti-AGEs activity of a wide variety of synthetic molecules has already been evaluated (Reddy and Beyaz, 2006), the chemodiversity of natural products such as secondary metabolites of vegetal origin still needs to be thoroughly explored (Dobson, 2004). Over the past decade, our laboratory was involved in phytochemical and biological studies of many species in the genera *Mesua* (Morel et al., 1999), *Garcinia* (Hay et al., 2004) and *Calophyllum* (Guilet et al., 2001; Hay et al., 2003). These plants belonging to Clusiaceae or Calophyllaceae families (The Angiosperm Phylogeny, 2009) share an original composition in polyphenol derivatives (*i.e.* benzophenones, biflavonoids, coumarins, naphtodianthrones, phloroglucinols, xanthones) (Filho et al., 2009; Su et al., 2008). This typical composition (Choi et al., 2008; Hanamura, 2005; Shao et al., 2008) thus prompted us to evaluate, using the recently developed procedure (Derbré et al., 2010), the anti-AGEs activity of crude extracts obtained from various tropical plants among these families.

2. Results

2.1. Anti-AGEs screening on vegetal extracts

The aforementioned anti-AGEs screening was conducted on 37 DCM and MeOH extracts prepared from various parts of 9 plants originating from Malaysia or New Caledonia and belonging to the Clusiaceae or Calophyllaceae families (Table 1). Among them, nine extracts showed an IC_{50} below 0.18 mg/mL corresponding to a five-fold increase in activity when compared with aminoguanidine (IC_{50} = 1 mg/mL), the usual reference. However, though aminoguanidine is an AGEs formation blocker used as reference compound in many studies (Lee et al., 2011; Tamarat et al., 2003), we have recently highlighted that simple flavonols such as quercetin appeared as much better AGEs inhibitors (Derbré et al., 2010). Therefore quercetin (IC_{50} = 0.18 mg/mL) was chosen as the second reference compound in our anti-AGEs assay. As a matter of fact the most potent activities were measured for DCM and MeOH extracts from *Mammea neurophylla* as well as MeOH extracts from *Garcinia vieillardii* bark and *Calophyllum flavoramulum* leaf. Immediately available and in sufficient amount, the Malayan and endemic *C. flavoramulum* Hend. & Wyatt-Sm. (Calophyllaceae) was then selected for phytochemical investigations (Ferchichi et al., 2009). This plant belongs to the genus *Calophyllum*, a large group of pantropical trees counting about 190 different species (Filho et al., 2009; Su et al., 2008). We thus embarked upon a bioguided fractionation of the MeOH leaf extract of *C. flavoramulum,* seeking for anti-AGEs natural compounds, whereas the cyclohexane, DCM and EtOAc extracts obtained from the same material were subjected to a systematic phytochemical study.

2.2. Bioguided fractionation procedure

Inactive (anti-AGEs assay) triterpenes commonly found in *Calophyllum* species such as canophyllol **6** and apetalactone **7** (Chart 1) were directly precipitated from the MeOH leaf extract of *C. flavoramulum* which was further fractionated to give ten fractions (I to X). The resulting fractions (Table 2) were then selected according to their anti-AGEs properties (*i.e.* $IC_{50} \leqslant 0.35$ mg/mL). Fraction II was fractionated using flash chromatography leading to sub-fractions exhibiting poor anti-AGEs activities, except for fraction II-15 with an IC_{50} of 0.014 mg/mL. This activity was attributed to the 3-methoxy-2-hydroxyxanthone **2** (IC_{50} = 0.06 mM). Fraction VI appeared as an excellent inhibitor of AGEs formation