ABSTRACT John Nii Adotey Addotey

Hyaluronic acid (HA) is an essential component of the extracellular matrix which is involved in several physiological and pathological processes. The concentration of HA depends on the dynamic equilibrium between the synthesis by hyaluronate synthase and the degradation by hyaluronidases. In somatic cells, Hyal-1 and Hyal-2 are mainly responsible for the breakdown of HA. Hyal-1 is a ubiquitous enzyme found in many tissues in the body and is implicated in a number of diseases such as bladder and prostate cancers. Hyaluronidase inhibitors have been investigated for use as contraceptives, wound-healing agents, antivenom and dermatological anti-ageing agents. Development of natural products as specific Hyal-1 inhibitors might be a promising lead for improved wound healing, tissue regeneration, and looking at renal function, for increased diuresis during urinary tract infections. This study sought to establish the pharmacological rationale for the traditional uses of the traditionally used herbal materials from Ononis spinosa L. (diuretic and anti-rheumatoid) and Phyllanthus muellerianus Kuntze Excell (anti-inflammatory and wound healing), based on inhibition of Hyal-1 by the respective plant extracts. Using HA as a substrate, it is possible to determine the activity of Hyal-1 which is expressed on the surface of Escherichia coli F470 cells. The stains-all assay enables the quantification of the undegraded HA. Hydroalcoholic extracts from the roots of O. spinosa showed moderate inhibiting effects against Hyal-1 (IC50 0.73 mg/mL), while the dichloromethane extract exerted an IC50 of 94 µg/mL.Bioassay guided fractionation of the dichloromethane extract yielded four isoflavonoids with anti Hyal-1 activity: onogenin, sativanone, medicarpin and calycosinD with relative inhibition rates of 25, 61, 22 and 23 %, respectively, at a test concentration of 250 µM. The IC50 of sativanone, the most active compound, was determined with 150 µM, which was better than that of the positive control glycyrrhizinic acid (177 µM). Thus, a possible explanation for the diuretic properties of O. spinosa root extract may be postulated from these results. The norneolignan clitorienolactone B, the first time described for the genus Ononis, was inactive, while the triterpene α-onocerine, the most abundant compound in the roots, had only limited inhibitory activity against Hyal-1.

However, clitorienolactone B and α-onocerine were found to exert anti-inflammatory effects against lipopolysaccharide stimulated neutrophils. This could justify the use of root extracts from O. spinosa roots in inflammatory conditions. Leaves and twigs from Phyllanthus muellerianus are known to exert anti-inflammatory and antipyretic properties as well as wound healing properties. A hydroalcoholic extract (PWE) exerted a concentration dependent inhibition of Hyal-1 with an IC50 of 80 µg/mL. Bioassay-guided fractionation revealed 13 compounds from the two most active fractions, which were mainly ellagitannins and flavonoid glycosides. The most active Hyal-1 inhibitor was found to be the ellagitannin chebulanin (IC50 132 µM), therefore, the antiHyal-1 activity observed for the extract can be correlated to this polyphenol. This represents the first description of chebulanin in P. muellerianus. Additionally, also synergistic effects were observed, indicating that the traditional use of aqueous extracts from P. muellerianus is justified, rather than the use of the isolated ellagitannins. The traditional use of the plant as an anti-inflammatory agent for improved wound healing can be rationalized by the anti-Hyal-1 activities of its constituents. In a pilot structure-activity study involving 13 flavonoid compounds, it became clear that flavonoids exhibit a structure dependent inhibitory activity against Hyal-1, qualifying them for further quantitative structure activity relationship studies.