

**Euro Pharmacognosy 2019: Qualitative Phytochemical Fingerprint and Network Pharmacology Investigation of Achyranthes aspera Linn. Extracts - Giustino Orlando-University "G. d'Annunzio" of Chieti-Pescara**

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**Short Communication**

**Abstract**

Achyranthes aspera Linn. (Amaranthaceae), commonly known as the Prickly Chaff flower, is used as herbal medicine in the Ivorian's culture, Africa. Nonetheless, there is currently a paucity of scientific information on A. aspera from the Ivory Coast. Herein, the antioxidant activity of A. aspera extracts (methanol, dichloromethane, ethyl acetate and infusion) as well as the enzymatic inhibitory potentials towards key enzymes in human diseases, namely Alzheimer's disease, (cholinesterases: AchE and BChE), type 2 diabetes ( $\alpha$ -glucosidase and  $\alpha$ -amylase) and hyperpigmentation (tyrosinase) were assessed. The total phenolic (TPC) and flavonoid (TFC) content was determined using colorimetric methods and the individual compounds were characterized using ultra-high performance liquid chromatography coupled with hybrid quadrupole-Orbitrap high resolution mass spectrometry (UHPLC-HRMS). Furthermore, a network pharmacology analysis was conducted to predict putative targets of identified phenolic compounds. The highest TPC was observed in the infused extract ( $28.86 \pm 0.12$  mg GAE/g), while the dichloromethane extract ( $38.48 \pm 1.48$  mg RE/g) showed the highest level of TFC. UHPLC-HRMS analysis has revealed an abundance of fatty acids, flavonoids, phenols and acylquinic acids. Among tested extracts, the infused extract displayed the highest free radical quenching, reducing and metal-chelating ability. The extracts (except infusion) were effective as enzyme inhibitors against AChE, while only methanolic and infused extracts showed noteworthy anti-BChE effects. The methanolic extract showed a remarkable antityrosinase effect ( $56.24 \pm 5.05$  mg KAE/g), as well. Modest to moderate inhibitory activity was observed against  $\alpha$ -amylase (all extracts) and  $\alpha$ -glucosidase (only dichloromethane extract). Finally, the network pharmacology analysis suggested the carbonic anhydrase II enzyme as a putative target for explaining, at least in part, the traditional use of A. aspera preparations as diuretic and blood clotting agent. Data amassed herein tend to validate the use of A. aspera in traditional medicine, as well as act as a stepping stone for further studies in the quest for novel phytopharmaceuticals. In this context, it is desirable that this study will contribute to the validation of the traditional uses of this plant in the African herbal medicine, and to the valorization of the whole chain production of A. aspera, as a local and sustainable botanical resource.

**Introduction**

The burden of non-communicable diseases (NCDs) is rising swiftly in low-resourced countries, resulting in ill health, worsened poverty and poor social development. In Sub-Saharan Africa, NCDs are the second most common cause of mortality, accounting for 2.6 million deaths annually, which is equivalent to approximately 35% of all deaths in the region Yuyun, et al. Healthcare systems in the majority of Sub-Saharan Africa countries are fragile, fragmented, under-resourced, inaccessible and inefficient for a quick and effective response to rising burden of NCDs and hence managing these chronic diseases in Africa represents a huge challenge. Thus, in line with the World Health Organization's strategy, healthcare authorities in many low-resourced countries have been promoting a form of healthcare system that combines both traditional practices, predominantly the herbal traditional medicine, and conventional medicine to alleviate diseases. As many African countries, traditional medicine is deeply rooted in the Ivorian culture, and has remained as the primary healthcare system. Among different medicinal plants, Achyranthes aspera L., is reputed for its use in the folkloric medicine of Ivory Coast.

**Results and Discussion**

Plants are considered as a repository of molecules with biological properties that are useful for the modern drug discovery program. Among the known classes of bioactive compounds, polyphenols are well acknowledged for their

potential as therapeutics. Therefore, the present study evaluated the total phenolic content (TPC) and total flavonoid content of *A. aspera* extracts using spectrophotometric methods. Experimental data expressed as equivalents of gallic acid (GAEs), for TPC, and rutin (REs), for TFC, are summarized. The TPC varied from  $14.28 \pm 0.24$  to  $28.86 \pm 0.12$  mg GAE/g, with the highest content observed in the infused extract. The dichloromethane extract ( $38.48 \pm 1.48$  mg RE/g) followed by ethyl acetate ( $29.90 \pm 0.71$  mg RE/g) extract displayed the richest total flavonoid.

Compound 1 yielded a deprotonated ion at m/z 187.096 (C<sub>9</sub>H<sub>16</sub>O<sub>4</sub>) together with the fragment ions at m/z 169.086 ([M – H – H<sub>2</sub>O]– and m/z 125.095 ([M – H – H<sub>2</sub>O – CO<sub>2</sub>] – suggesting carboxylic groups. This fragmentation pathway was previously described and 1 was identified as azelaic acid. In the same manner 2, 4 and 24 were tentatively identified as undecanedioic, dodecanoic and 9,10-dihydroxy-octadecanoic acids, respectively.

One carboxylic (quinic acid), five phenolic acids including three hydroxybenzoic acids (salicylic, protocatechuic and gentisic acid), three hydroxycinnamic acids including (caffein and ferulic acids), two monoacylquinic (chlorogenic and 4-caffeoylequinic acid), two diacylquinic acids (3,5-dicaffeoylquinic and 4,5-dicaffeoylquinic acids) and one triacylquinic acid were found in the studied *A. aspera* extracts. Moreover, two hexosides of salicylic and gentisic acids as well as six flavonoids were identified. Most of compounds were identified by comparison with standard references and literature data. The acylquinic acids elucidation was based on the structure-diagnostic hierarchical keys for the identification of chlorogenic acids, while flavonoid dereplication was supported by the RDA cleavages of the flavonoid skeleton.

### **Conclusion:**

Concluding, *A. aspera* extracts possess numerous secondary metabolites, including ferulic acid, apigenin and salicylic acid, with promising pharmacological applications in counteracting the burden of oxidative stress occurring in chronic inflammatory diseases, such as type 2 diabetes, cardiovascular and neurodegenerative disorders. On the basis of the present study, an improvement of the local chain production is desirable, also in view of more sustainable and circular economy.

**Note:** This work is partially presented in 7th European conference on Pharmacognosy, Medicinal Plants and Natural Products December 02-03, 2019 held at Paris, France.