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Short communication

ANASA - Development of bioactive herbal extracts to treat severe acute respiratory system diseases

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Introduction

Inhalation of plant extracts has proven to treat chronic or acute diseases of the respiratory tract, due to the presence of bioactive compounds with antioxidant, antimicrobial and anti-inflammatory activity that heal the bronchial epithelium (Santana et al., 2016). However, the protective role of inhalation of extracts derived from plants of the Greek flora in pulmonary inflammation is largely unknown. The purpose of "ANASA" is the development of an innovative inhaling device for the administration of extracts from Medicinal and Aromatic Plants (MAPs) cultivated based on organic farming practices and are included among the phytotherapeutic drugs of the European Medicines Agency (EMA). Specifically, we selected *Eucalyptus globulus*, *Calendula officinalis*, *Matricaria chamomilla*, *Mentha* X *piperita* Sm., *Rosa damascena*, and *Salvia triloba* and we hypothesized that inhalation of their extracts would exert anti-inflammatory effects on cigarette smoke-induced lung inflammation.

Materials and Methods

Organic Cultivation of MAPs

MAPs of Greek flora with an EU monograph by the Committee on Herbal Medicinal Products in the therapeutic areas of "cough and cold" and "pain and inflammation" were selected, and crops were established according to the organic farming practices. The quality production of the medicinal plants was ensured by the ideal location of "Aromafarms" farm in Peloponnese. The crops were grown under the supervision of the Organization for the Control and Certification of Organic Products.

Hydrodistillation and Extraction of Plant Material

For the quality control of the plant material, protocols for the receipt of volatile components (hydrodistillation (HD) of essential oils (EO)) and non-volatile components (extracts) were developed. The plants after drying were subjected to HD with a Clevenger apparatus. In parallel, the plant material was grinded and extracted by Ultrasound Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE) using water. To obtain enriched in phenols extracts, all the aqueous extracts that occurred after HD, UAE and MAE extraction were processed with adsorption macroporous resin FPX66.

GC-MS and HPTLC Analysis

Quality control of the plant material was carried out in two ways, for the volatile components a methodology was developed using GC-MS, while for the non-volatile

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components the chemical profile of extracts was characterized by HPTLC.

Antioxidant Capacity (TPC, DPPH)

The antioxidant activity of the extracts was evaluated by the DPPH assay while the total phenolic content (TPC) was determined using Folin-Ciocalteau method.

In-vivo model

Adult male C57BL/6 mice (n=10 per group) were exposed to cigarette smoke (CS, 3R4F) 4 times per day with 30 minutes intervals, for 3 consecutive days. Control animals inhaled atmospheric air. Aerosol from plant extracts (sage or rose) was administered to the mice 30 minutes prior to smoke exposure. Following 24 hours after the last smoke exposure, the mechanics of the respiratory system were measured, bronchoalveolar lavage (BAL) was performed to measure total cell count and protein levels in BAL fluid. Protein levels of Surfactant protein (Sp)-D, IL-6 and TNF-a were measured in BAL fluid by ELISA. Peribronchovascular and lung peripheral inflammation was also evaluated in lung tissue sections by histology.

Results and discussion

Quality Control of Plant Material - Characterization of Volatile and Non-volatile Content

To ensure superior plant quality to prepare extracts that are safe and effective, organic cultivations were established. One of the objectives was the comparative study of extracts from two growing seasons to ensure the supply of plant material of stable quality and quantity. The results from GC-MS were similar between the two crops and the EO contained, according to bibliography, the main constituents. The HPTLC analysis revealed the compound categories, mainly flavonoids and phenolic constituents (UV abs. and brown-yellow spots after spraying with the sulfuric vanillin), phenylethanoid derivatives (UV abs. and red staining spots after spraying). Terpene derivatives (blue spots with the appearance reagent) were also detected.

Total Phenolic Content (TPC) and DPPH Assay

The highest phenolic load was presented by mint, sage, rose and eucalyptus. Chamomile and calendula did not appear to contain rich phenolic content. Moreover, it was revealed that enriched extracts after treatment with resin (HD-XAD), gave extracts with richer phenolic content than the preparation of infusions with HD. The highest antioxidant capacity was shown by sage, mint, rose and eucalyptus while chamomile and calendula were characterized by moderate and low activity, respectively. This is in line with the higher phenolic load of the respected extracts. Comparing the antioxidant capacity of plants, it was observed that sage and rose have high antioxidant activity, while comparing the various extracts with each other, it was revealed that HD decoctions and enriched HD-XAD extracts showed strong antioxidant capacity as well.

In-vivo Model

Following cigarette smoke (CS) exposure, total cell count in BAL fluid was significantly increased ($p<0.05$ to control). Inhalation of rose extract did not affect BAL cellularity. In contrast, sage extract inhalation ameliorated the increase in BAL cell count ($p=0.006$ to CS). Interestingly, both rose and sage inhalation inhibited IL-6 and TNF-a increase in BAL fluid after CS exposure. CS caused only a modest increase in respiratory system compliance ($p<0.05$ to control), an effect that was not influenced by sage or rose inhalation. Total protein in BAL fluid, SpD protein levels in BAL fluid and histology were not affected by CS exposure.

Conclusion

Selected MAPs were cultivated, extracted and their chemical content was characterized. Moreover, their TPC content was evaluated along with their scavenging capacity against the free radical DPPH. The most promising extracts, *R. damascena* and *S. triloba*, were further studied in *in-vivo* models to evaluate whether they exert anti-inflammatory effects on cigarette smoke-induced lung inflammation. Results revealed that a short course of CS inhalation induces pulmonary inflammation that is mainly prevented by sage extract inhalation.

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