NEWSLETTER N°3
April 2022

IUPHAR Mediterranean Group
of Natural Products Pharmacology

Membrane-interactive compounds from *Pistacia lentiscus* L. thwart *Pseudomonas aeruginosa* virulence

COMMENTED BY Céline Rivière, Joint Research Unit 1158 BioEcoAgro, Univ. Lille, INRAE, Univ. Liège, UPJV, YNCREA, Univ. Artois, ULCO, F-59650 Villeneuve d'Ascq, France

AUTHORS Ali Tahrioui[1], Sergio Ortiz[2], Onyedikachi Cecil Azuama[1], Emeline Bouffartigues[1], Nabiha Benalia[2], Damien Tortuel[1], Olivier Maillot[1], Smain Chemat[3], Marina Kritsanida[2], Marc Feuilloley[1], Nicole Orange[1], Sylvie Michel[2], Olivier Lesouhaitier[1], Pierre Cornelis[1], Raphaël Grougnet[2], Sabrina Boutefnouchet[2], Sylvie Chevalier[1]

AFFILIATION Academic: [1] Laboratoire de Microbiologie Signaux et Microenvironnement, LMSM EA4312, Université de Rouen Normandie, Normandie Université, Évreux, France.
[2] CiTCoM UMR 8038 CNRS, Faculté des Sciences Pharmaceutiques et Biologiques, Équipe Produits Naturels, Analyses et Synthèses (PNAS), Université Paris Descartes, Paris, France.
[3] Centre de Recherche Scientifique et Technique en Analyses Physico-Chimiques, CRAPC, Bou Ismaïl, Algeria.

JOURNAL AND BIBLIOGRAPHIC REFERENCES Frontiers in Microbiology (2020) 11: 1068. doi: 10.3389/fmicb.2020.01068. PMID: 32528451

STUDY DESIGN Preclinical *in vitro* study

TREATMENT *Pistacia lentiscus* fruit cyclohexane extract and ginkgolic acids

Main results and author's conclusion

The authors of this paper describe the *in vitro* anti-virulence potential of an apolar extract of *Pistacia lentiscus*, and some of its components, against *Pseudomonas aeruginosa*. *P. lentiscus* L. fruits cyclohexane extract (PLFE1) showed a statistically significant and dose-dependent inhibition of pyocyanin production (IC50 = 4.9 µg mL–1), without affecting the growth of the bacteria and cell viability. Pyocyanin production inhibition correlates with the reduction of the expression levels of some genes involved in phenazine-1-carboxylic acid biosynthesis and in its conversion in pyocyanin. Moreover, the virulence of *P. aeruginosa* in human lung A549 cells and in *Caenorhabditis elegans* infection models is attenuated by PLFE1. This extract also interferes with *P. aeruginosa* quorum-sensing molecules production. Finally, PLFE1 increases the membrane stiffness in P. aeruginosa by decreasing the expression of the extra-cytoplasmic function sigma factor (ECFσ) SigX, which is involved in the regulation of membrane lipid composition and may have an impact on direct or indirect regulation of the expression of genes involved in the production of pyocyanin and *P. aeruginosa* quorum-sensing molecules. To conclude, two major components of the apolar extract, ginkgolic acid (C17:1) and hydroginkgolic acid (C15:0), were identified as being responsible for the main anti-virulence activity against *P. aeruginosa*.

Commentary

P. aeruginosa is an environmental bacterium, which is well-known due to its adaptation abilities and its involvement in numerous chronic and acute life-threatening infections as a multidrug resistant opportunistic Gram-negative bacterium. In acute infections, *P. aeruginosa* deploys a panel of virulence factors, among which pyocyanin, a redox-active, phenazine-derived blue-green pigment promoting the colonization and the dissemination of the bacterium. The development of anti-virulence agents is an alternative to fight against *P. aeruginosa*. *P. lentiscus* L. belonging to *Anacardiaceae*, commonly known as mastic tree, is a widely distributed shrub in the Mediterranean basin and whose uses have been known since antiquity. Thereby, the present study suggests that the anti-virulence effect of an apolar extract of *P. lentiscus* and some of its metabolites, that are gingkolic acid analogs, could be due to their interaction with the lipid bilayer membranes of P. aeruginosa, resulting in the diminution of membrane fluidity. The increased membrane stiffness

appears to be mediated through the modulation of the ECFσ SigX, that contributes to the regulation of *P. aeruginosa* virulence. Further experimental data will allow to confirm the specific molecular mechanisms of action of this extract and its metabolites.

Petasin and isopetasin reduce CGRP release from trigeminal afferents indicating an inhibitory effect on TRPA1 and TRPV1 receptor channels

COMMENTED BY Stefania Ceruti, Dipartimento di Scienze Farmacologiche e Biomolecolari – Università degli Studi di Milano, Milano

AUTHORS Kleeberg-Hartmann Johanna, Vogler Birgit, Messlinger Karl

AFFILIATION Academic: Institute of Physiology and Pathophysiology, Friedrich-Alexander-University of Erlangen-Nürnberg, Universitätsstraße 17, 91054 Erlangen, Germany

JOURNAL AND BIBLIOGRAPHIC REFERENCES J Headache Pain (2021) 22(1): 23.
https://doi.org/10.1186/s10194-021-01235-5

STUDY DESIGN Preclinical *in vitro*

TREATMENT Extract and petasin/isopetasin

Main results and author's conclusion

Common butterbur (*Petasites hybridus*), belonging to the *Asteraceae* family, grows domestic in Europe (in the Alps and Apennine environment) and North-Western Asia and its alcoholic root extract is utilized in migraine prevention. The analgesic activity of butterbur extract has been long linked to the presence of two sesquiterpene derivatives, namely petasin and isopetasin, but their molecular targets and mechanisms of action are not clear. In this paper, the Authors have evaluated the effects of a butterbur alcoholic root extract, or of petasin and isopetasin alone, on the release of CGRP, one of the most important mediators of migraine attacks, from meningeal afferents and trigeminal ganglia preparations in vitro. CGRP release was stimulated through the activation of TRPA1 and TRPV1 receptors (which are known to be involved in various types of pain, not only in the head and face districts) with mustard oil and capsaicin, respectively. Results show that butterbur extract, petasin or isopetasin as inhibited TRPA1- and TRPV1-mediated CGRP release.

Commentary

The interest in the identification of the molecular and cellular mechanisms at the basis of the pharmacological effects of plant extracts traditionally utilized in therapy is growing over years and is becoming increasingly important. This is due in part to the request for "natural" therapies from patients, but mostly to the need to better understand how the potential health benefits of these natural preparations can be fully exploited, by limiting side effects and drug interactions. Despite the lack of a clear dose-response correlation and the need of additional studies, the results of this paper highlight a new mechanism of action of butterbur extract. Moreover, data suggest that both TRPA1 and TRPV1 are likely involved in the development of migraine pain, and demonstrate an important link between the analgesic activity of butterbur and the CGRP signaling system. The latter represents one of the most studies mechanisms at the basis of migraine and it is the target of the most recent approved anti-migraine drugs, including several monoclonal antibodies.

Physicochemical characteristics and antiproliferative and antioxidant activities of Moroccan Zantaz honey rich in methyl syringate

COMMENTED BY Nour Eddine ES-SAFI, Mohammed V University in Rabat

AUTHORS Youssef Elamine[a,b,c], Badiaa Lyoussia, Maria G. Miguelc, Ofélia Anjos[d,e,f], Letícia Estevinho[g], Manuel Alaiz[b], Julio Girón-Calle[b], Jesús Martín[h], Javier Vioque[b]

AFFILIATION Academic/Industry: [a]Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life (SNAMOPEQ), University of Sidi Mohamed Ben Abdellah, Fez 30000, Morocco; [b]Instituto de la Grasa (C.S.I.C.), Universidad Pablo de Olavide, Edificio 46, Carretera de Utrera, km 1, 41013-Sevilla, Spain; [c]Mediterranean Institute for Agriculture, Environment and Development, Universidade do Algarve, Faculdade de Ciências e Tecnologia, Edificio 8, Campus de Gambelas, 8005-139 Faro, Portugal; [d]Instituto Politécnico de Castelo Branco, 6001-909 Castelo Branco, Portugal; [e]Centro de Estudos Florestais, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal; [f]Centro de Biotecnologia de Plantas da Beira Interior, 6001-909 Castelo Branco, Portugal; [g]Centro de Investigação de Montanha, Instituto Politécnico de Bragança, 5300-252 Bragança, Portugal; [h]Fundación MEDINA, Avda del Conocimiento 34, 18016-Granada, Spain.

JOURNAL AND BIBLIOGRAPHIC REFERENCES Food Chemistry, Volume 339, 1 March 2021, 128098. https://doi.org/10.1016/j.foodchem.2020.128098

STUDY DESIGN Preclinical *in vitro* study

TREATMENT Six Zantaz honey samples produced from the melliferous plant *Bupleurum spinosum*

Main results and author's conclusion

The authors present a study about the phytochemical composition of six honey samples, locally known as Zantaz honey, which is produced by a melliferous plant, *Bupleurum spinosum* of the *Apiaceae* family. The antiproliferative and antioxidant activities of the investigated honey samples were further explored. Results showed that the phenolic composition of the honey samples, explored through HPLC, was characterized by the presence of methyl syringate as major compound, exceeding 50 % of the total polyphenols content. Other phenolic compounds such as epicatechin, syringic acid and catechin were also detected.

The antioxidant and antiproliferative activities were investigated using Caco-2 and THP-1 cells. Results showed that the six honey samples exhibited antioxidant activity and inhibited cell proliferation. Positive correlation was observed between the biological activity and the content in methyl syringate and gallic acid.

Commentary

Honey is a bee product which has been used as food and in healthcare since ancient times. Its uses as a remedy against various pathologies dates back hundreds, even thousands of years ago. As a natural product, it is considered as safe for use and with few side effects. Over the years, the virtues of honey have attracted the interest of modern medicine and researchers. Nowadays, the interest of the scientific community toward honey is much more increasing, in the light of its strong potential biological activities. Despite the work carried out on honey, this field remains an open research area, able to highlight differences due to the different types of pollen gathered by bees.

The work published by Elamine et al. reports results dealing with the phytochemical composition and the antioxidant and antiproliferative activities of Zantaz honey. Results provide new information allowing to discover the benefits of this type of honey produced from *Bupleurum spinosum*, a shrub that grows mainly in the Atlas Moroccan Mountains The presence of methyl syringate as major phenolic compound, in addition to the positive correlation between the observed activities and the content in methyl syringate and gallic acid, are reported.

Probiotics-Containing Mucoadhesive Gel for Targeting the Dysbiosis Associated with Periodontal Diseases

COMMENTED BY Giuseppe Giannini, R&D Alfasigma S.p.A., Via Pontina Km. 30.400, 00071 Pomezia (Roma), Italy

AUTHORS Giuseppe Giannini[1], Irene Ragusa[2], Giulia Nerina Nardone[2], Sara Soldi[3], Marina Elli[3], Piera Valenti[4], Luigi Rosa[4], Emanuele Marra[5], Daniela Stoppoloni[5], Emilio Merlo Pich[6]

AFFILIATION Industrial: [1]R&D Alfasigma S.p.A., Via Pontina Km. 30.400, 00071 Pomezia, Roma; [2]Labomar S.p.a., Via F. Filzi 33, 31036 Istrana, Treviso; [3]AAT – Advanced Analytical Technologies Srl, Via P. Majavacca 12, 29017 Fiorenzuola d'Arda, Piacenza; [5]Takis s.r.l, Via di Castel Romano 100, 00128, Roma; [6]R&D Alfasigma S.p.A., Via Ragazzi del '99, n°5, 40133 Bologna.
Academic: [4]Department of Public Health and Infectious Diseases, University of Rome La Sapienza, Piazzale A. Moro 00185 Roma.

JOURNAL AND BIBLIOGRAPHIC REFERENCES International Journal of Dentistry, 2022, 16 pages, ID 5007930, https://doi.org/10.1155/2022/5007930

STUDY DESIGN Preclinical *in vitro/in vivo* study

TREATMENT Association of selected probiotics and botanical extract in a slow-release lipogel oral formulation.

Main results and author's conclusion

The main oral disorders are associated with forms of oral dysbiosis that favour the development of periodontitis (pyorrhoea), a chronic multifactorial inflammatory disease that results in the progressive destruction of the supporting structures of the teeth, namely periodontal ligament, and alveolar bone. Possible consequences are tooth loss in adults and a risk factor for systemic complications, including cardiovascular and dysmetabolic diseases. From a etiological point of view, in a variable time frame, the normal bacterial flora, consisting of Gram-positive species, is progressively substituted by a pathogenic "red complex", consisting mainly of facultative intracellular anaerobic pathogens such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, *Tannerella forsythia*, and *Prevotella melaninogenica*. An innovative mucoadhesive gel-based formulations (AL0005) has been now developed, which can be loaded with selected probiotics and botanicals, ensuring their localized (oral mucosa) and prolonged (up to 8 hours) release. The formulation is strictly anhydrous, to prevent bacterial growth, and could found application in topical treatments to prevent and cure oral cavity pathologies as mucositis, ulcers, gingivitis, tooth decay and periodontitis.

Commentary

Oral diseases are widely distributed worldwide with nearly 3.5 billion people interested (Lancet 2018, 392, 1789–8583). As co-author of the paper and inspirator of this project, I can say that this formulation represents a significant step forward in the treatment of oral dysbiosis, in a non-aggressive and natural way. Contrary to the products currently available on the market, which are based on formulations that release a probiotic in few minutes, this formulation is able to release, locally and slowly (up to 8h), consortia of probiotics combined with botanical extracts, individually known and tested to be effective in ensuring oral eubiosis. Formulation is stable if left at room temperature for at least one year, an essential requirement for its commercialization. Optimization of this formulation is currently underway to make it available on the market by the end of the year.