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CORRESPONDENCE/REBUTTAL

Comment on Comparison of Protective Effects between Cultured *Cordyceps militaris* and Natural *Cordyceps sinensis* against Oxidative Damage

In a recent paper published in this Journal, Yu et al. (*1*) compared the in vitro antioxidative properties of *Cordyceps militaris* and *Cordyceps sinensis*. The paper reported that the radical scavenging activitiy was, at least in part, due to the presence of flavonoids and other polyphenolics, and the authors concluded that these extracts might have beneficial "effects in human diseases related to oxidative stress" such as atherosclerosis and Alzheimer's disease. The experimental data and some of the conclusions need a critical comment.

The fruiting bodies of entomopathogenic ascomycetes of the genus *Cordyceps* together with the attached host larvae are highly valued drugs in traditional Chinese medicine. In particular, natural Dong Chong Xia Cao (*Cordyceps sinensis* with its host *Hepialus armoricanus*) is extremely rare, highly valued for its purported properties, and, therefore, highly priced. Natural *C. militaris* is similarly not readily available and rather costly. Thus, a growing number of so-called *Cordyceps* products that derive from mycelial cultures of the asexual forms of these fungi have become commercially available.

A first problem with the published paper stems from the fact that it lacks any information concerning the origin and taxonomic characterization of the fungal strains. Appropriate characterization of test material is essential, because the outcome of the biological tests critically depends on the strains used. According to molecular phylogenetic studies, *Cordyceps* does not represent a single evolutionary lineage (*2*), and accurate taxonomy is thus essential. *Cordyceps* cannot be cultured as such, but only as their anamorphs, which are assigned to the genera *Paecilomyces*, *Hirsutella*, *Hymenostilbe*, *Verticillium*, and *Nomurea* of the deuteromycetes (*3, 4*). Hence, the term "cultured *Cordyceps sinensis*" is misleading and should be replaced by a correct taxonomic assignment.

A second problem relates to the insufficient characterization of the chemical composition of the investigated samples. For the cultured sample, exact fermentation conditions are lacking. Secondary metabolite expression in fungal cultures critically depends on medium composition, harvest time, temperature, and other fermentation parameters, which, therefore, must be published. Furthermore, adequate analysis of the chemical composition of all extracts is needed and should, at least, consist of a gradient HPLC fingerprint covering the entire spectrum of metabolites, plus a specific dosage of major and/or pharmacologically relevant classes of compounds. Without this information, published biological data simply cannot be reproduced by other scientists. The authors provide a HPLC fingerprint of nucleosides recorded under isocratic conditions. However, these compounds represent only a fraction of the metabolite spectrum of a crude extract. Compound classes that are critical in the context of the published paper, such as the phenolic constituents, are not analyzed by HPLC.

An obsolete and nonspecific colorimetric assay is used for a quantitative determination of radical scavenging phenolics. According to the paper, these phenolics should consist mainly of flavonoids. However, the reasons for assuming the presence of flavonoids are not given. Up to now, flavonoids have been generally considered as a compound class that occurs exclusively in higher plants (*5*). Scattered reports on flavonoids in fungi have been poorly documented (*6, 7*), and, even though Kitamoto and co-workers recently reported the occurrence of type III polyketide synthase genes including chalcone synthase-like genes in *Aspergillus oryzae* and some other deuteromycetes (*8, 9*), the activities of these genes remain unknown. Given that, conclusive evidence would be needed to support the presence of flavonoids in a fungal extract.

A possible, and much more plausible, explanation for the outcome of the colorimetric assay (and for the observation of radical scavenging activity) is the occurrence of pyridone alkaloids and tetramic acids in the extracts. These pigments are responsible for the characteristic yellow-orange color of the fruiting body of *C. militaris*, and we have identified such pigments, which we named militarinones, in several strains of its anamorph, *Paecilomyces militaris* (*10, 11*). These metabolites also possess the necessary structural features to provide a positive color reaction of "flavonoids" in the colorimetric assay. Given the structures of the militarinones, one can also reasonably assume that they possess radical scavenging properties. Indeed, we found some, albeit weak, radical scavenging activity in mycelial extracts of *P. militaris* strains (*12*).

A third point relates to overinterpretation of biological/pharmacological data. The title "Comparison of Protective Effects ... against Oxidative Damage" is imprecise and misleading. Oxidative damage of what? The wording of the title implicitly suggests some in vivo study, or at least a complex cellular model. The paper, however, describes only simple cell free in vitro assays such as scavenging of the $ABTS^{\bullet+}$ radical, albumin, and LDL oxidation. The published paper attempts to construct a link between epidemiological studies on dietary phenolic intake, the importance of radical species in chronic

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diseases, and beneficial effects due to intake of *Cordyceps* and contains statements such as "In other words, CME (*Cordyceps militaris* extract) and CSE (*Cordyceps sinensis* extract) that protect LDL against oxidation may help to prevent atherosclerosis and coronary heart disease." Suggesting, on the basis of results from an in vitro test with LDL particles, that *Cordyceps* extracts should have a preventive effect against atherosclerosis and coronary heart disease is scientifically not admissible. One cannot extrapolate to a possible clinical benefit on the basis of preliminary in vitro data. The same applies for albumin oxidation and the link to a protective effect on Alzheimer's disease. Imprecise and vague statements such as the final sentence of the paper, "Therefore, additional consumption of CME and CSE may increase the levels of phytochemicals (comment: which phytochemicals, and where should they be increased?) and enhance therapeutic effects (comment: therapeutic effects of what should be enhanced?) in human diseases related to oxidative stress", should be avoided.

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