Year
2017

- [2018](#)
- [2017](#)
- [2016](#)

Issue

- [03: e82-e113](#)
- [02: e43-e81](#)
- [01: e1-e42](#)
- [S 01: GA 2017 – Book of Abstracts](#)

- [Table of Contents](#)
- [Current Issue](#)

Zu den Ausgaben VOR Heft 2/2016

- [Planta Medica Letters](#)

Related Journals

- [Planta Medica](#)
- [Drug Research](#)
- [Pharmacopsychiatry](#)
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PMIO 2017; 4(S 01): S1-S202

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Evaluation of antioxidant potential and cytotoxic effects of alcoholic extracts of *Diospyros kaki* leaves

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[Further Information](#)

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- [Full Text](#)

Diospyros kaki (Dk) leaves have been used in China, Korea and Japan as a delicate, pleasant beverage. Additionally, it is used as a folk remedy for hypertension, ischemic stroke, angina and internal hemorrhage. However, in EU and particularly in Portugal Dk leaves are considered an agriculture waste. The aim of this study was to characterize methanolic Dk leaf extract concerning its in vitro antioxidant proprieties and evaluate eventual cytotoxic effects, using HepG2 cells (hepatocytes). HPLC-DAD profiles of Dk leaves showed that extracts are mainly composed by phenolic compounds (flavonoids and phenolic acids derivatives), known antioxidants. To assess antioxidant potential, we used several in vitro tests including DPPH scavenging, iron chelation, inhibition of nitric oxide and superoxide scavenging. Results showed that Dk have a high capacity to scavenge superoxide radicals (EC_{50} 8.94 $\mu\text{g/ml}$), even better than quercetin (EC_{50} 18.73 $\mu\text{g/ml}$), and DPPH radicals (EC_{50} 15.27 $\mu\text{g/ml}$). For iron chelating activity and inhibition of nitric oxide production Dk extract had lower potential with and EC_{50} of 1376 and 1027 $\mu\text{g/ml}$, respectively. So, we can conclude that Dk leaf extract is a good radical scavenger and might protect cells from oxidative stress. Since Dk leaves are used in beverages and liver is always a key target organ for metabolism and detoxification, we tested possible cytotoxicity on HepG2 cells. Results did not show significant cytotoxic effects of Dk extracts on HepG2 cells (using MTS evaluation), up to 1 mg/ml, for 3 and 24h incubation times. Hence, we can infer that Dk leaf extracts have good antioxidant potential and no significant toxicity (at least on hepatocytes), however more tests are required with other cellular models.

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Top of Page](#)