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SHORT COMMUNICATION

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In vitro and *in vivo* studies of *Cucurbita pepo* L. flowers: chemical profile and bioactivity

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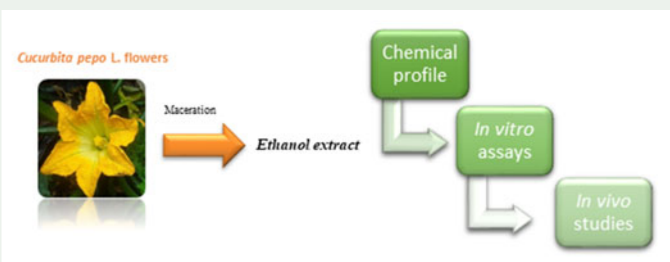
ABSTRACT

Edible flowers consumption has increased in recent years due to their rich content of healthy phytochemicals. The aim of this study was to analyse the chemical profile of *Cucurbita pepo* L. flowers, and to explore their antioxidant and hypoglycaemic properties. Moreover, in order to assess *in vivo* effects, biochemical analysis, Reactive Oxygen Metabolites (d-ROMs) and Biological Antioxidant Potential (BAP) tests were performed on mice serum. High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD) analyses revealed the presence of (+)-catechin, (−)-epicatechin, rutin, and syringic acid as main constituents. 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP) tests showed interesting results. The extract exhibited the strongest inhibitory effect on α-glucosidase (IC_{50} of 144.77 μg/mL). *In vivo* results confirmed the hypoglycaemic effects, also affecting lipid metabolism but did not revealed benefits on ROS production. These results may add some information supporting the use of *C. pepo* flowers as functional foods and/or nutraceuticals.



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1. Introduction

There is a renewed interest for the use of edible flowers not only for aesthetic value but also for healthy properties. This renewed interest has increased the research on their chemical profile and bioactivity (Mlcek and Rop 2011; Loizzo et al. 2016). The Cucurbitaceae family consists of about 965 species in around 98 genera. One of the most widely known species is *Cucurbita pepo* L. The delicate texture and slightly sweet flavour of *C. pepo* flowers have made them one of the favourite ingredients. These flowers are a rich source of healthy constituents and are used in traditional medicine for their anti-inflammation, anti-diabetic, and anti-hypercholesterolemic properties (Gutierrez 2016). The objectives of this study were to investigate the chemical composition of *C. pepo* flowers by HPLC-DAD and to validate the effectiveness as potential functional foods and/or nutraceutical ingredients by investigating the antioxidant properties, the inhibitory activity of α-amylase and β-glucosidase, and biochemical parameters *in vivo*.

2. Results and discussion

The ethanol extract of *C. pepo* flowers showed a total phenols content of 98.63 mg of chlorogenic acid equivalents/100 g fresh weight (FW) and a total carotenoids content of 55.27 mg of β-carotene equivalents/100 g FW were found. Twenty compounds were used for HPLC-DAD analyses (Table S1, supplementary material) that revealed the presence of (+)-catechin, (−)-epicatechin, rutin, and syringic acid as dominant compounds. Values of 22.74 and 35.32 mg/100 g FW were found for hesperidin and quercetin-3-*O*-glucoside, respectively. *C. pepo* extract was tested by using 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Ferric Reducing Antioxidant Power (FRAP), 2,2-diphenil-1-picrylhydrazyl (DPPH), and β-carotene bleaching tests (Table S2, supplementary material). The most interesting results were obtained in FRAP and ABTS tests. Despite the results obtained *in vitro*, *C. pepo* did not influence oxidative biomarkers in mice, neither the BAP nor the d-ROMs values, probably due to the chosen of the extract dose administered to the mice. Reactive Oxygen Species (ROS) react with every organic molecule they meet, producing reactive oxygen metabolites (ROMs), which are more stable than the ROS and are therefore easier to quantify. Conversely, the biological antioxidant potential (BAP) matches the total antioxidant capability of plasma and includes either exogenous or endogenous components that can oppose the oxidant action of reactive species (Lombardi et al. 2017; Morittu et al. 2018). The extract of *C. pepo* was investigated for its potential hypoglycaemic (Table S3, supplementary material). The strongest inhibitory activity was obtained against α-glucosidase (IC_{50} of 144.77 μg/mL). Some of the most abundant identified compounds previously showed good hypoglycaemic activity (Tundis et al. 2016).

Mice body weight (BW) significantly increased along the time ($p < 0.0001$; Figure S1, supplementary material) but was not influenced by the extract. Average daily gain (ADG) was similar among groups and no significant interaction was observed for both BW and ADG (Figure S2, supplementary material). Similarly, feed and water intake (Figure S3, supplementary material) were not different among groups and by time and the interaction Group x Time was also not significant. Biochemical parameters and oxidative status are showed in Table S4, supplementary material. Among blood chemistry parameters, statistical differences were registered for glucose (GLU) ($p = 0.0250$), urea (UREA) ($p = 0.0103$), CPK ($p = 0.0066$), and cholesterol (CHOL) ($p = 0.0004$) values. In particular, C-high group showed values of GLU, creatine-phosphokinase (CPK), and CHOL lower than control, while C-low administration reduced only UREA and CHOL levels respect to control group. The administration of *C. pepo* extract did not influenced the oxidative status of mice. Few studies are reported in literature concerning the *in vivo* the effects of *C. pepo*. Kaur et al. (2017) found a significant increase of body weight in diabetic rats treated with 100, 200 and 400 mg/kg of *C. pepo* seeds extract, and this increase was dose-dependent.

Our results obtained *in vivo* confirm the potential hypoglycaemic activity of *C. pepo* in a dose-dependent way. Nowadays, therapeutic targets for diabetes look for a long-lasting maintenance of glycaemic control and numerous plants, including *C. pepo*, were found to be effective in the long-term management of type 2 diabetes mellitus (Gutierrez 2016). Xia and Wang (2006) evidenced how *C. pepo* extract reduces blood glucose levels and increase insulin levels in streptozotocin induced diabetic rats and hypothesized that the glycaemic control was due a potentiation of plasma insulin effect as consequence of an increase of insulin secretion or its release from the bound form. More recently, Mahmoodpoor et al. (2018) showed that administration of 5 g lyophilized pulp powder decreased both blood glucose level and insulin dose. Sedigheh et al. (2011) found a significant decrease in glucose, triglycerides, and low-density lipoproteins (LDL) after 4-weeks daily administration in male diabetic rats treated with 1 g/kg of pumpkin powder. In another study, Gourgue et al. (1992) showed that pectin, considered as a hypoglycaemic factor, is abundant in *C. pepo*. Even if mechanisms are still unknown, soluble dietary fiber seem to affect the nutrient transit rate into the gut, thus reducing glucose absorption post-prandial glycaemia and consequently enhancing the glycaemic control (Musco et al. 2016). The significant decrease in cholesterol level observed in male mice orally treated with 100 and 200 mg/kg Live Weight (LW) is in agreement with Sedigheh et al. (2011) who used pumpkin powder of *C. pepo*. Indeed, the soluble fibre was reported to decrease cholesterol levels in both blood and muscle (Musco et al. 2015). Linoleic acid and oleic acid, fatty acids identified in *C. pepo* seed, reduced cholesterol levels in rats (Takada et al. 1994). Ziaee et al. (2009) demonstrated that rutin at 100 mg/kg alone or in combination with lovastatin was able to reduce the levels of cholesterol and LDL and to decrease liver enzymes and weight in animals with a high-cholesterol diet. CPK levels are highly variable between mice, for this reason data is often limited to comparison of levels within a study and not absolute values between studies (Spurney et al. 2009). Since the other muscle markers (lactate dehydrogenase, LDH, and aspartate aminotransferase, AST) did not show statistical differences, and CPK levels are also highly influenced by multiple factors (De Luca et al. 2005), the lower values of CPK needs further studies to be elucidated.

3. Conclusion

Flowers consumption has significantly increased in recent years due to their content of healthy constituents. Herein, we analysed the chemical profile of *C. pepo* flowers, and we explored their antioxidant and hypoglycaemic properties. (+)-Catechin,

(−)-epicatechin, rutin, and syringic acid were identified as main constituents. The hypoglycaemic effects of *C. pepo* extract obtained *in vitro* are confirmed *in vivo*. The extract also affected *in vivo* lipid metabolism but did not reveal benefits on ROS production. Finally, obtained results highlighted *C. pepo* flowers as a food aimed at satisfying both taste and health.

Disclosure statement

No conflict of interest was reported by the authors.

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