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To cite this article: Essam Abdel-Sattar, Azza Ramy Abdel-Monem, Mohamed Elamir F. Hegazy, Ali M. El-Halawany & Sherif Mahmoud Afifi (2021) Genetic diversity, LC-ESI-MS chemical profile and *in vivo* antitumor activity of three Egyptian soybean cultivars, Natural Product Research, 35:1, 135-139, DOI: 10.1080/14786419.2019.1610955

To link to this article: https://doi.org/10.1080/14786419.2019.1610955
SHORT COMMUNICATION

Genetic diversity, LC-ESI-MS chemical profile and in vivo antitumor activity of three Egyptian soybean cultivars

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ABSTRACT

Soybean (\textit{Glycine max} L.) is one of the most important and widely consumed food plants worldwide. The objective of this study was to investigate the metabolite profiling of three Egyptian soybean cultivars (Giza 22, Giza 35 and Giza 111) and their in vivo antitumor effect. Random amplified polymorphic DNA (RAPD) analysis developed polymorphism level of 75\% in 72 distinct markers. Applying LC-ESI-MS analysis, twenty-nine metabolites were recognized from the 80\% methanol extract of all cultivars. In vivo antitumor activity of the 80\% methanolic extract against solid Ehrlich ascites carcinoma (EAC) inoculated in mice model, showed a significant diminishing in tumor volume and reduced Glutathione (rGSH) and a significant increase in malondialdehyde (MDA) which was supported by histopathological examination. Among the studied cultivars, Giza 22 cultivar contained the highest total phenolic content (TPC) that may contribute to its impressive antioxidant capacity and antitumor activity.
1. Introduction

Cancer (also known as malignant neoplasm or tumor) is not a single disease but involves a set of diseases in which abnormal cells divide in an uncontrolled manner, and it may occur in various parts of the body (McCracken and Phillips 2012). Experimentally, some herbal components and medicinal plants have been reported to possess promising anticancer activity (Nussbaumer et al. 2011).

Commercially, soybean is cultivated today mainly for its protein and oil components. The frequencies of some cancer types, especially colon, breast, and prostate are significantly lesser in countries that include soybean in their diets (Liss et al. 2010; Applegate et al. 2018). Soybean products have acquired special interest due to their role in treating and protection against cancer in vitro (Marrelli et al. 2013); however, it is obvious that the in vivo results are not sufficient to conclude the part that soybean consumption plays in cancer (Nakamura et al. 2011). Hsu et al. (2010) showed that soy extract induced more apoptosis in prostate carcinoma cells as compared to purified genistein or daidzein. In addition, they suggested that individual isoflavones may have higher cytotoxicity in non-cancerous cells. Plants that contain polyphenolic compounds are thought to have vital role as antioxidant for the prevention of free radical-associated diseases including cancer (Kurutas 2016). LC–MS/MS analysis has been successfully used for the structural elucidation of phenolic compounds in various samples (Zunjar et al. 2015). The aim of our research is to determine genetic variation and relationship among three Egyptian soybean cultivars, in relation to their total phenol, antioxidant activity and metabolites profile using LC-ESI-MS analysis and to evaluate their therapeutic values as antitumor activity in animal model.

2. Results and discussion

2.1. RAPD technique for the analysis of soybean cultivars

The fingerprints (Figure S1) with various band sizes of PCR markers ranged from 230 to 1700 bp revealed 72 total scorable bands with an average amplification number of 7.2 per primer among which 54 were polymorphic (Table S1). The highest percent (88.89%) of polymorphic bands was found in primers OPA-1 and OPA-9 while primer OPA-5 generated the lowest percent (50%). It is interesting to consider the relatively high polymorphism percent (75%) that is supported by other studies of genetic diversity on soybean cultivars (Bisen et al. 2015; Bharose et al. 2017). Giza 35 and Giza 111 were closely related while Giza 22 with similarity index 0.7077 indicated a larger genetic distance (Figure S2).

2.2. Chemical profile analyses of soybean cultivars using LC-ESI-MS

For the phytochemical analyses of soybean (Figure S3), LC-ESI-MS analysis in both positive and negative ionization modes were utilized. The identity of 29 metabolites present in all cultivars (Table S2 and Figure S4) was ascertained using data from LC-ESI-MS analysis by comparison and combination of chromatographic behavior.
matching with standards and mass spectra (fragment ion values, MS and MS/MS). The identified metabolites include four phenolic acids (two hydroxybenzoic acid derivatives and two hydroxycinnamic acid derivatives), twelve isoflavones (three aglycones and nine glucosides), twelve soyasaponins (three group A soyasaponins, four group B soyasaponins and five DDMP-conjugated saponins) and one anthocyanin. The isoflavones were the predominant class of phenolics in Giza 22 cultivar. Genistein content was quantified to be $83 \pm 0.08$, $12.3 \pm 0.05$, and $55.8 \pm 0.11$ mg/100 g soybean on dry weight bases in extracts of Giza 22, Giza 35 and Giza 111, respectively.

### 2.3. Total phenolic content (TPC) and antioxidant activity

The TPC of the 80% methanol extracts of the investigated three cultivars, was as follows; Giza 22, Giza 35 and Giza 111 with $695 \pm 12$, $580 \pm 16$, and $525 \pm 10$ mg GAE/100 g dry soybean, respectively. Considering the correlation between total phenolic contents and antioxidant effects, a direct proportional association was realized (Figure S5).

### 2.4. Evaluation of in vivo antitumor activity

Soybean extracts at doses of 90 and 50 mg/kg showed a significant reduction in tumor volume (Figure S6) and reduced Glutathione (rGSH) (Figure S7) and a significant increase in malondialdehyde (MDA) (Figure S8) and supported by the histopathological examination (Figure S9). Generally, it can be pointed out that Giza 22 extract at a dose of 90 mg/kg was the most effective extract with results comparable to doxorubicin group.

Most of the identified compounds from LC-ESI-MS analysis are belonging to the isoflavones and phenolic acid derivatives. There is an increasing interest in the biological effects of phenolics, since there are numerous studies connecting phenolic content with antioxidant and anticancer activities (Bursal et al. 2013). The antioxidant and cytotoxic effects of hydroxyflavones were related to the number of hydroxyl groups and their position in the molecule (Kilani-Jaziri et al. 2012; Kumar and Pandey 2013). An association between antioxidant activity and inhibition of proliferation was found by Mfotie Njoya et al. (2017) who reported a positive correlation between inhibition of four human cancer cell lines (MCF-7, HeLa, Caco-2 and A549 cells) by *Sarcocephalus pobeguinii* extracts and their antioxidant activities against DPPH, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and nitric oxide. Further, the oxidative stress is considered as cancer initiation and thus potent antioxidants could be a strategy to combat progression of carcinogenesis. The role of antioxidant as an antitumor agent depends on its potential as an oxygen radical inhibitor (Kumar and Pandey 2013). The findings of this study showed that all three cultivars have reasonable and variable antioxidant and antitumor activities with respect to their chemical profiling and the major identified compounds from HPLC-ESI-MS analysis.
3. Conclusion

We believe that the synergistic effect of some major individual compounds such as p-hydroxybenzoic acid and genistein confirmed by HPLC-ESI-MS analysis in addition to other phenolic acids and isoflavones may be responsible for antioxidant properties and anticancer activity of 80% methanol extract of soybean.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or non-profit sectors.

Disclosure statement

The authors declare that there is no conflict of interest regarding the publication of this article.

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