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Effects of gamma irradiation and comparison of different extraction methods on sesquiterpene lactone yields from the medicinal plant *Thapsia garganica* L. (Apiaceae)

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ABSTRACT

Ethnopharmacological relevance: *Thapsia garganica* L. roots are used in Algerian traditional medicine for a number of ailments. It is used in a poultice as an antitussive treatment of acute bronchitis and pneumonia, in preparations with milk or oil taken orally to treat common lung diseases, and with the direct application of root sections for the soothing of dental pains.

Aim of the study: The objective of this study was to evaluate the combined effect of microwave assisted extraction and gamma irradiation on sesquiterpene lactones in *T. garganica* extracts

Materials and methods: To evaluate the combined effect of microwave assisted extraction and gamma irradiation on the highly bioactive compounds found in extracts of Algerian *T. garganica*, samples from different locations in Algeria were prepared by extraction from dried leaf and root samples of dried plant material, using different extraction methods Quantification of the compounds of interest was done using an HPLC. The antioxidant activity extracts was determined using the two free radical scavenging assays: the 2,2-diphenyl-picryl-hydrazyl (DPPH) and the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS).

Results: It was found that location and extraction method had significant impact on the phytochemical composition of extracts. Gamma irradiation was found to have no effect on the phytochemical composition of the plant extracts or on their antioxidant properties.

Conclusion: The study has shown that microwave assisted extraction is an effective method for investigating chemical compounds in *T. garganica* and the results support the notion that gamma irradiation for sterilisation do not alter the chemical composition.

The authors wish to clarify that we cannot recommend the usage of any parts of *T. garganica*, in any form, for any remedy due to its very high toxicity.

1. Introduction

1.1. Traditional use in Algeria

Thapsia garganica L. (Apiaceae) is a medicinal plant commonly found in Algeria, along the coast, in the plains, in the Saharan Atlas Mountains and in the north of the Saharan desert (Hammiche et al., 2013). It is commonly referred to as: Toufelt in Berber; adhriss by the Kabyle people in the North; thapsie, bounafaa or bou-nafit «that of efficacy» in Arabic; faux fenouil (false fennel) and Thapsia du mont Gargan in French(Hammiche et al., 2013). In English, it is known as the deadly carrot. All parts of the plant are known to be toxic and irritant to the skin, causing blisters, erythema and itching, and the resin of the roots has been found to be particularly toxic (Andersen et al., 2015b).

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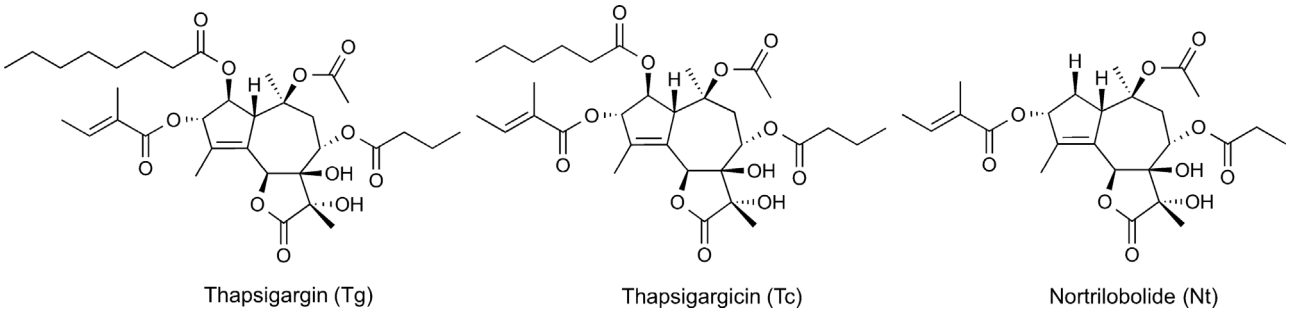

Fig. 1. Illustration of structures of the three main sesquiterpene lactones in *Thapsia garganica* L. Thapsigargin (Tg), thapsigargicin (Tc), and nortrilobolide (Nt).

Due to this toxicity *T. garganica* is not allowed in any official pharmaceutical preparation, and we cannot recommend the usage of any parts of *T. garganica*, in any form, for any remedy due to its very high toxicity. *T. garganica* roots are still used in Algerian traditional medicine for a number of ailments. In Kabylia, the Kabyle people use the root to make a "depurative cure" at the onset of spring (Hammiche et al., 2013). They also use the roots to make a poultice, which is applied to the chest as an antitussive treatment of acute bronchitis and pneumonia. Great care is taken in the preparation and its use is limited; in fact, it is a treatment of last resort when bad weather prevents travel (Hammiche, 2015). If the medical condition is less severe, the oil in which a fresh root is cooked is either rubbed on the chest for its "purgative" properties or ingested in small quantities (Hammiche, 2015). Other traditional uses in Algeria include a preparation with milk or oil taken orally to treat common lung diseases, and the soothing of dental pains with the direct application of root sections (Hammiche et al., 2013).

The toxicity of *T. garganica* originates from the presence of thapsigargin (Fig. 1) and other sesquiterpene lactones (Andersen et al., 2015a, 2017; Drew et al., 2009; Simonsen et al., 2013). Thapsigargin makes up 0.2–1.2% of the dry weight of the plant's roots (Andersen et al., 2015b). The pharmacological activity of thapsigargin is due to its inhibition of the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) in mammalian cells, which leads to cell apoptosis (Simonsen et al., 2013).

1.2. Antioxidant activity

Both the food and pharmaceutical industries have shown a continuing interest in finding naturally occurring antioxidants for use in the preservation of foods or medicinal products, in order to replace synthetic antioxidants, which are being restricted due to their carcinogenicity and harmful effects on the environment (Prakash et al., 2015). Essential oils from aromatic and medicinal plants, in particular, have been of special interest due to their strong antioxidant activity and antimicrobial constituents in their tissues (Di Venere et al., 2016; Golubović et al., 2014). It has previously been seen that certain Algerian medicinal plants, including *T. garganica*, contain strong radical scavengers and can therefore be useful as sources of natural antioxidants for both medicinal and commercial use (Djeridane et al., 2006). However, as many of these plants contain toxic compounds, toxicity issues need to be addressed to ensure the antioxidants are safe to use.

1.3. Irradiation of medicinal herbs

Medicinal plants are widely used in Algerian folk medicine, especially by the elderly and rural communities with limited access to doctors. However, the plants are subject to deterioration from chemical and microbial processes that occur before reaching the end-user during harvesting, processing, distribution and storage. These processes can alter their efficacy and in some cases their safety, so there is a demand for methods of decontamination and preservation in order to improve consumer safety and therapeutic efficacy. Food irradiation is commonly used to sterilise and to reduce food losses due to spoilage, and it has replaced once commonly used chemical fumigants, like ethylene oxide, and other chemical preservatives that have been reported to be hazardous to human health (Seo et al., 2007). The use of gamma irradiation on food products is approved by the Food and Agriculture Organisation (FAO), the International Atomic Energy Agency (IAEA) and the World Health Organisation (WHO)(Joint, 2009). It has been shown to be a safe, environmentally friendly and energy efficient method to sterilise plant products. It is also a well-established industrial process for the sterilisation of medicinal plants in a number of facilities worldwide and in general do not affect the chemical composition of the leaves and roots (Garg and Gupta, 2016; Seo et al., 2007).

1.4. Extraction methods

Traditional extraction methods of medicinal plants include decoction or maceration in an organic solvent. These methods however, are highly energy dependent and time consuming. Microwave assisted extraction (MAE) has been found to be a reliable alternative as it requires a lower energy input to result in the same or even higher extraction yields, reduces the use organic solvents, shortens extraction times and improves the reproducibility of results. This extraction method has been used for the analysis of bioactive compounds in a number of medicinal plants (Akloul et al., 2014; Benkaci-Ali et al., 2006; Kennouche et al., 2015). However, care should be taken to choose suitable conditions to avoid the thermal degradation of the analytes of interest. Sample preparation and extraction methods are important to consider when studying medicinal plants, as the methods chosen depend on the target compounds and can affect the phytochemical composition of the final extracts.

Here we investigate the chemical composition of extracts of *T. garganica* from different regions in Algeria. We evaluate the combined effects of microwave assisted extraction and gamma irradiation on the extraction yield of the bioactive compounds as well as on the antioxidant activity of the extract.

2. Materials and methods

2.1. Plant material

Thapsia garganica L. (Apiaceae) roots and leaves were collected between March and April in 2014 and 2015 during flowering, from two locations in Algeria: Médea (Aïn Boucif) (GPS coordinates N35° 53′ 28″/E3° 9′ 31″) and Béjaia (Kherrata) (GPS coordinates N36° 29′ 34″/E5° 16′ 39″). At each site 50 individuals were sampled. For each individual representative leaf material was taken across the entire plant and roots were dug out. Herbarium vouchers were made for one individual per site. The herbarium vouchers are deposited at the Natural History Museum of Denmark, Herbarium C (C10011584, C10011585; leg. Abir Mohamed Ibrahim). The local name, the used plant parts, methods of preparation and administration, and medicinal uses were collected from local inhabitants. Samples were identified Dr. Abdelkrim