FTIR spectroscopic analysis and effect of Diplotaxis Acri’s flower extract on pro-inflammatory cytokines in THP-1 human macrophages

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ABSTRACT
Plants and natural compounds are extensively reported for diverse biological activities, including their effects on the inflammation pathway. The annual winter herb Diplotaxis Acris (D.Acris) is found only slopes of sandy and stony valleys in the desert. This research is intended to make a contribution to the literature regarding the employment of the species of plant ethnomedicinally by undertaking FTIR spectroscopic analysis and examining several concentrations of the plant’s extract for anti-inflammatory activity in vitro with activated THP-1 human macrophages to examine its mediating effect on inflammation. Cell viability was also evaluated, and there was no severe cytotoxic effect from D.Acris extract with any of the concentrations being assessed on cells. ELISA was used to assess the pro-inflammatory cytokines and chemokines that were produced. It has been noted that the plant extract led to a significant decrease in levels of the pro-inflammatory cytokines, including interleukin (IL)-1β, tumour necrosis factor (TNF)-α and IL-6. Inhibition of pro-inflammatory cytokines indicates the anti-inflammatory trends of D.Acris. This plant can be investigated further by isolation of natural compounds from the extract and effects of these compounds can be evaluated on the same inflammatory markers to show the main active constituent responsible for anti-inflammatory activities.

INTRODUCTION
Diplotaxis DC is a genus of Brassiceae, the most valuable (economically) branch of the Brassiceae family (Hussein et al., 2017). There are between 27 and 36 species, and their habitat ranges from the Mediterranean areas of Europe to Northwest India (Larsen and Boulos, 1999). The winter herb Diplotaxis Acris (D. acris), Figure 1 commonly called salad rocket (Grillo et al., 2012), grows on the slopes of sandy and stony valleys in the desert; (Oueslati et al., 2015) it is similar in taste to Eruca Sativa Mill. (Osman et al., 2019) and favoured by grazing animals (Shaye et al., 2020). As well as being economically vital, the plant has antioxidant properties (Atta et al., 2004). Locally (in Saudi Arabia) named as gahag, it is an annual glabrescent plant. Identification of the plant is possible through its fleshy leaves and substantial purple flowers (Al-Jaber et al., 2011).

Chemically analyzing the plant demonstrated that it holds several chemical compounds including quercetin 7-rhamnoside-3’-methyl ether, kaempferol 3-O-glucoside, luteolin 7-rhamnoside, isorhamnetin 3-O-glucoside, luteolin 7-diglucoside, apigenin, apigenin 7-diglucoside and quercetin (Hussein et al., 2017). Clinical studies also showed that fatty acids are present alongside...
sterols such as β-sitosterol, α-linolenic acid, and benzyl benzoate; alkaloid choline chloride is also present (Hussein et al., 2017). Additionally, with doses between 200 and 400 mg/kg D.Acris was shown to have peripheral and central antinociceptive activity, thus giving it antioxidant properties (Awaad et al., 2011) demonstrated that the high segments of D.Acris have anti-diarrheal properties, shown from an evaluation of diarrhoea induced by castor oil, rat gastrointestinal movement, and newly slaughtered rabbit duodenal motility. Doses between 200 and 400 mg/kg have been shown to have a notable anti-diarrheal influence on diarrhoea induced by castor oil in rats (Atta et al., 2005).

As far as can be ascertained, no previous detailed mechanistic research has been undertaken into the anti-inflammatory properties of the D.Acris flower; in the light of new global initiatives to discover new sources of medicines and food, this research intends to review and analyze the pharmacological properties of this plant in terms of its ability to act as an anti-inflammatory.

Cytokines are elemental to chronic inflammatory disease pathogenesis (Calamia, 2003), with several factors, including oxidative stress, modulating their secretion (Taylor et al., 2004). Tumour necrosis factor-alpha (TNF-α) is a cytokine that is multifunctional (Aggarwal, 2000), regulating the way activated leukocytes grow, reproduce, are differentiated, and become viable (Dempsey et al., 2003). This cytokine is also a trigger for cells to release other cytokines, chemokines, and inflammatory mediators (Hsu et al., 1995); it has demonstrable antiviral and antimicrobial properties (Wajant et al., 2001). This research is intended to examine the direct influence of the extract of the D.Acris flower concerning pro-inflammatory cytokine secretion.

**MATERIALS AND METHODS**

**Plant material/extract preparation**

Flowers from the D.Acris plant were harvested from the northern Saudi Arabian region of Aljouf in March 2019 (springtime). Once the flowers were harvested, a Soxhlet extractor was used with ethanol solvent. The process continued until all colours had bled into the solvent.

**Analysis of plant extract on Fourier Transform Infrared Spectrophotometer (FTIR)**

FTIR may be the most potent form of identification for chemical bonds (functional groups) within compounds. The chemical bond has a character-
FTIR spectral data interpretation

Figure 2 shows the FTIR spectrum for flower extracts that underwent preparation in ethanol of D. acris. Table 1 shows the data regarding peak values and likely functional groups (found through FTIR analysis) existing in the ethanol-prepared flower extracts of D. acris.
For the evaluation of the cytotoxic effects of D.Acris extract, the macrophages underwent stimulation using a variety of extract concentrations, with or without LPS. There was no severe cytotoxic effect from D.Acris extract with any of the evaluated concentrations, whether in basal conditions (A) or inflammatory conditions (B).

Effect on LPS activated THP-1 macrophages

For the investigation of the influence of D.Acris extract in terms of modulating inflammatory responses, ELISA was used to analyze pro-inflammatory cytokine levels. When LPS was not present, cytokine levels were around those of the controls Figure 3. As predicted, adding the pro-inflammatory stimulus of LPS led to a significant increase in the levels of all cytokines. Nevertheless, if the cells were cultured with D.Acris extract present (10 μg/mL ethanolic extract), the rise in cytokine levels was significantly reduced, with the values obtained being on a par with those found in basal conditions Figure 4.

CONCLUSION

The results of this research lead to the conclusion that D.Acris extract and its phytoconstituents can operate as a provider of immunomodulators. The different functional groups found within the extract appear to show that cellulose, glycogen, lipids, phosphates, amides, amino acids, glycogen, carotenoid, and carbohydrates can be present in this plant. One of the functional groups that were found in the extract was the OH group, which has the capability of forming hydrogen bonding capacity; this group’s presence in the D.Acris ethanol extract is likely to indicate that the extract has excellent potential for the inhibition of inflammatory activities.

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CONFLICT OF INTEREST

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