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SHORT COMMUNICATION

Echinophora tenuifolia L. branches phytochemical profile and antiproliferative activity on human cancer cell lines

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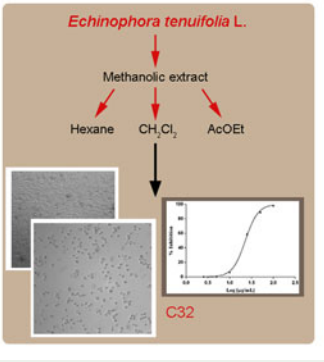
ABSTRACT

The methanolic extract of *Echinophora tenuifolia* L. branches and its fractions were evaluated for their *in vitro* cell growth inhibitory activity on different human cancer cell lines (C32, LoVo and SKBr3) and the normal BJ fibroblasts. All tested samples were effective against the melanoma cell line C32, with IC_{50} values ranging from 22.8 ± 0.8 to 78.7 ± 1.2 μg/mL, the antiproliferative activity of the dichloromethane fraction being significantly higher. This fraction was also effective against the LoVo adenocarcinoma cell line, with an IC_{50} value of 53.0 ± 2.1 μg/mL. The ethyl acetate and dichloromethane fractions showed the highest lipid peroxidation inhibitory activity, verified by means of the β-carotene bleaching test. The phytochemical profiles of *E. tenuifolia* branches extract were established by means of GC-MS and HPTLC. Overall, branches of *E. tenuifolia* L. could represent a rich source of bioactive compounds, potentially useful in the pharmaceutical field.



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1. Introduction

The *Echinophora* genus (Apiaceae) includes several species, distributed in Mediterranean and Middle East regions. In Italy *E. tenuifolia* is present in Puglia,

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Basilicata, Calabria and Sicilia (Pignatti 1982). *Echinophora* species were traditionally utilized for the treatment of wounds and gastric ulcers due to their antifungal and digestive properties (Glamočlija et al. 2011). Some of these species are added to soup, meat, cheese and yoghourt, to enhance their sensory properties, and the essential oil of *E. platyloba* DC has been recently used as a natural preservative in dairy products industries (Telci and Hisil 2008; Gokbulut et al. 2013). This genus has not yet been fully investigated. The most studied species are *E. platyloba* DC. and *E. sibthorpiana* Guss (Gholivand et al. 2011). The phytochemical profile of *E. cinerea* (Boiss.) Hedge *et* Lamond has been also reported (Jelodarian et al. 2017). We recently demonstrated the *in vitro* anti-inflammatory activity of inflorescences from *E. tenuifolia*. The methanolic extract and its fractions were demonstrated to inhibit the nitric oxide production in LPS-stimulated RAW 264.7 macrophages (Marrelli et al. 2017). To the best of our knowledge, relatively few studies have investigated the potential anticancer activity of *Echinophora* species, the only species studied being *E. Platyloba* DC. (Birjandian et al. 2018). Given these encouraging data, the aim of the present study was to evaluate *(i)* the antiproliferative activity of *E. tenuifolia* L. branches against human cancer cell lines; and *(ii)* its metabolites profiles and antioxidant potential.

2. Results and discussion

Branches from *E. tenuifolia* L. were collected in the province of Crotone in Calabria (Italy) in September 2010. A voucher specimen is deposited in the Herbarium of the University of Calabria (CLU 21879, leg. and det. C. Gangale, D. Uzunov). Two terpenoids were identified in *n*-hexane fraction (Table S1), the monoterpenes *o*-cymene and cumic alcohol. Fatty acids were more abundant, with linoleic acid as the major constituent (7.8% of total peak areas in TIC), followed by myristic acid (2.9%) and 10,13-octadecadienoic acid (2.3%). Moreover, some interesting phytosterols were also recognized, stigmasterol being the most abundant (3.8%). The analysis of the dichloromethane fraction allowed the identification of the monoterpenoid phenol carvacrol (5.0%, Table S1). The more polar ethyl acetate fraction was analyzed by HTPLC, which allowed to tentatively identify the flavonoid glycoside rutin (36.5 ± 1.9 mg/g of fraction). The chromatographic profiles of sample and standard (Rf = 0.13) are reported in Figure S1. Total phenolics and total flavonoids contents of the raw extract of *E. tenuifolia* L. branches were also assessed and were equal to 3.8 ± 1.5 mg/g and 0.14 ± 0.01 mg/g, respectively.

The crude extract showed a modest capacity to scavenge DPPH (IC_{50} = 360.9 ± 13.2 μg/mL) but was effective in protecting linoleic acid from peroxidation with an IC_{50} value of 24.4 ± 1.0 μg/mL after 30 min incubation, even if this effect significantly decreased after 60 min (IC_{50} = 50.5 ± 1.5 μg/mL) (Table S2). The highest radical scavenging potential was observed for the ethyl acetate fraction, with an IC_{50} value equal to 53.7 ± 1.5 μg/mL. The same sample showed also the strongest capacity to protect linoleic acid from peroxidation, as assessed by the β-carotene bleaching test. After 30 min of incubation, an IC_{50} of 15.6 ± 0.3 μg/mL was measured. After 60 min, the antioxidant potential of this fraction decreased but was still interesting (IC_{50} = 31.8 ± 0.9 μg/mL). The dichloromethane fraction exhibited also a capacity to

inhibit lipid peroxidation, with IC_{50} values of 22.7 ± 1.3 and 46.8 ± 1.5 μg/mL after 30 and 60 min, respectively. The activity of these two fractions can be related to the presence of phenolic compounds, notably the flavonoid glycoside rutin and the monoterpenic phenol carvacrol (Yang et al. 2008; Yanishlieva et al. 1999). We recently reported the antioxidant potential of *E. tenuifolia* L. inflorescences (Marrelli et al. 2017). The crude inflorescences extract showed a better radical scavenging activity compared to branches, with an IC_{50} value of 84.5 ± 2.6 μg/mL; also for this plant part, the ethyl acetate and dichloromethane fractions were the most effective samples in protecting linoleic acid from peroxidation.

The antiproliferative activity of *E. tenuifolia* L. branches extract and fractions was assessed *in vitro* on three different human cancer cell lines, the colorectal adenocarcinoma LoVo, melanoma C32 and breast cancer SKBr3 using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The effects on viability of normal BJ fibroblast cell line were also verified. As illustrated in Figure S2, all the samples significantly inhibited cell proliferation of cancer cells compared to control (as evidenced by Dunnett's multiple comparison test). The raw extract and fractions were effective against melanoma cell line C32, with percentages of inhibition ranging from 55.6 ± 0.7 to 97.8 ± 0.9%. The dichloromethane fraction induced 97.8 ± 0.9% of inhibition of melanoma cancer cells and 83.5 ± 3.8% of inhibition against LoVo cancer cells. This sample was effective also against breast cancer cells SKBr3, but at a lower inhibition level (37.48 ± 1.09%). Interestingly, *E. tenuifolia* extract and fractions showed no effects against the normal human fibroblast BJ cell line, with the only exception of the dichloromethane fraction (6.2 ± 0.6% inhibition at 100 μg/mL). *E. tenuifolia* extract and fractions were then tested at different concentrations, in order to estimate the IC_{50} values (Table S3). The inhibitory activity of the dichloromethane fraction was significantly higher than those of other samples, with an IC_{50} value equal to 22.8 ± 0.8 μg/mL on melanoma cancer cell line C32 (Figure S2). The dichloromethane fraction was effective also against the LoVo adenocarcinoma cell line, with an IC_{50} value of 53.0 ± 2.1 μg/mL (Table S3). All other tested samples were able to inhibit C32 cells viability, with IC_{50} values ranging from 70.0 ± 1.0 to 78.7 ± 1.2 μg/mL (Table S3). The antiproliferative activity exerted by the dichloromethane fraction could be related to the presence of carvacrol. This monoterpenoid phenolic is known for its antiproliferative activity against different human cancer cell lines (Yin et al. 2012; Mehdi et al. 2011). This compound showed also effectiveness against the colon adenocarcinoma LoVo cells (Fan et al. 2015). The promising results regarding the dichloromethane fraction, particularly active against both C32 and LoVo cell lines, suggest the need for further research to identify active principles with potentially interesting antiproliferative activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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