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Current approaches and challenges for chemical characterization of inhibitory effect against cancer cell line isolated from Gokshur extract

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ABSTRACT

In the present study, the potential effect anti tumor and the chemical composition of different fractions of Gokshur was evaluated. Commonly known as puncture vine, it has been used for a long time in both the Indian and traditional Chinese medicine. It is popularly used as a remedy for fertility disorder in Ayurveda. Samples were collected during June–September 2014 in the Cap Bon (north east of the northern Tunisia). Different organs were separated and extracted by sequential process to compare and investigate their potential anti-tumor effect. For the first time, we report the antiproliferatif effect of leaves *n*-butannolic fraction against cancer cell lines. The better anti-tumor fraction (94.76 ± 1.52%) has been detected and performed by RP-HPLC has shown a great peak area (5578.21 Mau). Novel designed natural derivatives from Gokshur, a cyclotrisiloxane, major compound identified by GC–MS.

1. Introduction

Ovarian cancer is the fifth most important cause of cancer-related mortality in females and is a lethal disease among all gynecologic malignancies. The main difficulty in arduous ovarian cancer is that the majority of patients are examined at an advanced stage. According to prior studies, as many as 70% of patients have already accessed stages III or IV of the ailment at the time of diagnosis [1]. Due to the latent beginning of ovarian cancer, no effective medical screening process has yet been showed for the mended identification and detection of the disease. In addition, the current approach and clinical therapy for ovarian cancer is optimal primary cytoreductive surgical operation, followed by systemic chemotherapy coupled to paclitaxel and platinum [2]. However, due to the side effects of this medicinal alternative and the high tolerance of ovarian cancer to chemotherapy, the efficiency of chemotherapy is limited. Ayurveda and Traditional Chinese medicine (ATCM) have been used in China and India for 1000 years as an antitumor treatment for a number of different malignancies, including lung, liver, and hematopoietic cancers [3–5]. Recently, ATCM are considered to induce few side-effects and little tumor cell resistance and have been known as a key source of novel drugs for targeted therapies. Clinical practices application have also shown that a number of Traditional Chinese medicine (TCMs) exhibit antitumor activity, which supplies a modern therapeutic strategy for cancer treatment.

Tribulus terrestris L. (TT) commonly known as Gokshur or Gokharu or puncture vine [6] has been used for a long time in both the Indian and Chinese systems of medicine [7], to treat various kinds of diseases. TT natural products perform various functions and many of them are interesting and useful biological activities [8–14]. TT growing in Bulgaria is a source for the industrial production of the original preparation "TribestanTM" produced by Sopharma AD, Bulgaria. TribestanTM consists of the *n*-butannolic (*n*-BuOH) extract of the aerial parts of the same plant and is successfully applied for treatment of sexual deficiency [15]. The active components of TribestanTM are steroid saponins of furostanol type 2–4. The dominating furostanol bis glycosides have been identified as protodioscin and protogracillin. An intensive screening on qualitative and quantitative composition of raw materials from TT and variety of preparations from different origin demonstrated that

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Bulgarian preparation TribestanTM contains the highest amount of protodioscin and protogracillin. The aphrodisiac property of TT extract was explored in castrated mice [16]. Administration of TT to humans and animals improves spermatogenesis. Most of the chemical investigations described in the literature refer to TT of Chinese, Indian and Bulgarian origin [17]. Despite the great pharmacological profile assigned to this herbaceous, there are limited studies of secondary metabolite in the same plant species from other countries and no study from samples growing in Tunisia flora exept a study developped by Bouabdallah [18]. Molecular identification with precise information on the composition of complex natural extracts (metabolomes) that are derived from medicinal plants is a challenging task that requires sophisticated, advanced analytical methods. In this respect, significant advances in hyphenated chromatographic techniques reversed phase high pressure liquid chromatography (RP-HPLC) and liquid chromatography–mass spectrometry (LC–MS), as well as data mining and processing methods, have occurred together, these tools, in combination with bioassay profiling methods, serve an important role in metabolomics for the purposes of both peak detection, quantification and identification in natural product research.

In the present study, a survey of the techniques that are used for generic and comprehensive profiling of secondary metabolites in natural extracts from TT is provided. The various approaches chromatographic methods: RP-HPLC; gas chromatography–mass spectrometry (GC–MS), are discussed with respect to their resolution and sensitivity for extract profiling. In addition in vitro cytotoxic activities of TT on various human ovary tumor cell lines have been examined. So our study report for the first time the best antiproliferatif fraction against tumor's IGROV (IGROV-1 human ovarian cancer) and OVCAR (OVCAR-3 human ovarian cancer) cell line with respectively ($88.19 \pm 0.88\%$; $94.76 \pm 1.52\%$) inhibitory activities. Furthermore, this high antiproliferatif effect derived from leaves *n*-BuOH fraction has been detected by RP-HPLC process with great peak area (5578.21 Mau) and identified using a GC–MS finger print.

2. Experimental

2.1. Reagents

Analytical grade ethanol, chloroform and *n*-butannol obtained from Merck (Nottingham, UK), all reagents were purchased from Sigma–Aldrich-Fluka (Saint-Quentin, France). Murine macrophage cell line RAW 264.7 (ATCC, TIB-71) was used in this study. For the chemicals, thiazolyl blue tetrazolium bromide [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT), isopropanol, sodium dodecyl sulfate (SDS) and all reagents used for cell culture were purchased from Sigma (St. Louis, MO USA), Fluka Chemie (Buchs, Switzerland) and Merck (Nottingham, UK).

2.2. Collection of plant material

Samples of TT were collected from plants grown in the region of Elhawaria (Boukrim) (Fig. 1a–c). Samples collection (June–September 2014) was conducted during the period of young leaves stage, flowering stage, mature fruit stage (when the fruits were ripening) Harvested plants were identified according to Pottier-Alapetite [19]. Furthermore confirmed to previously described methods by our Colleagues Botanists (INRAT, ENAT). Voucher specimens were deposited in the herbarium of our laboratory for future reference. Leaves, fruits, stems and roots were separated and dried at room temperature under dark conditions prior to use.

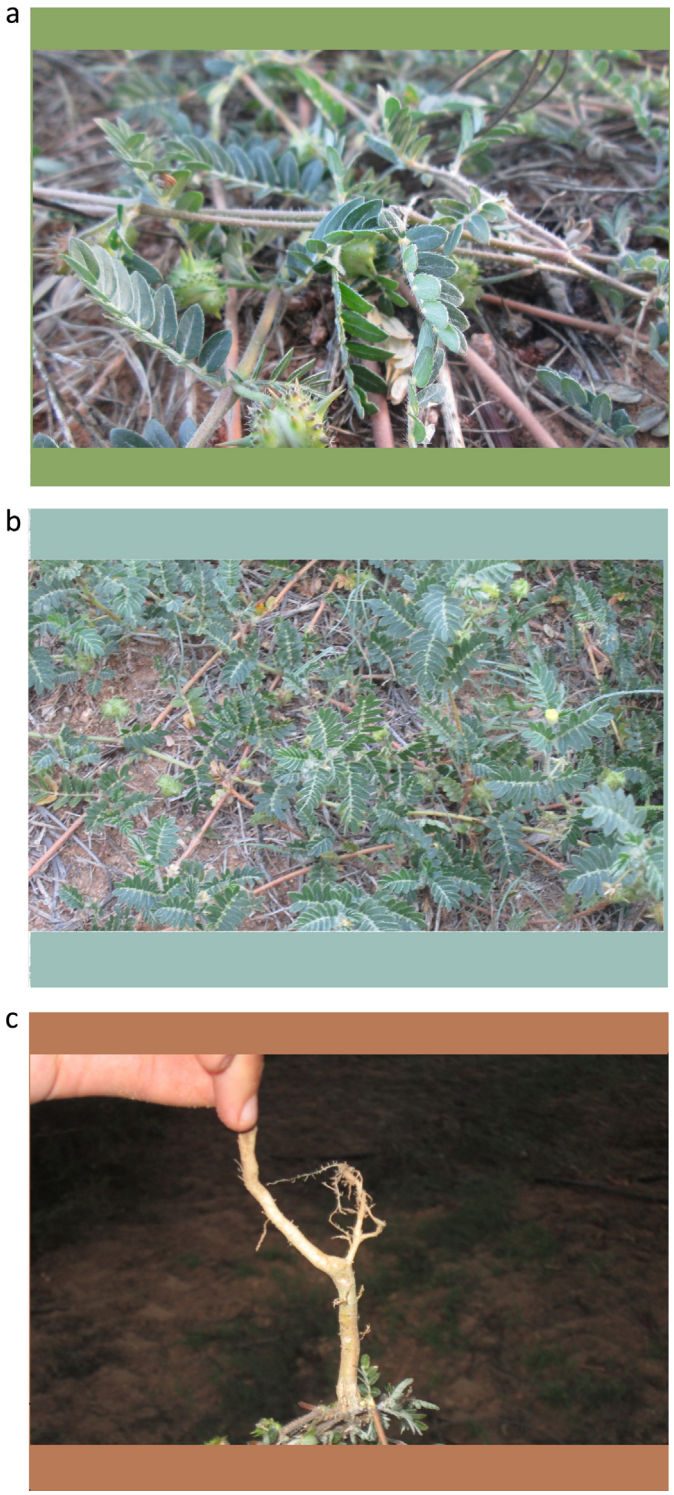

Fig. 1. *Tribulus terrestris* Linn. (Gokshura) samples growing spontaneously in the north east of northern Tunisia (El hawaria: Boukrim Ain halloufa).

2.3. Preparation of sample solutions by conventional heat reflux extraction

The dried and powdered plant material (powder:30 g) was extracted in a succession by chloroform at room temperature (3×270 mL $\times$ 1 h) and 70% v/v ethanol (reflux at 80 °C, 3×450 mL $\times$ 2 h). The combined ethanol solutions were concentrated under vacuum at 70 °C to a small volume ~150 mL and extracted in the separator funnel with *n*-butanol (3 times 60, 45,