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Antiproliferative activity of *Saponaria vaccaria* constituents and related compounds ☆,☆☆

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ABSTRACT

Total methanolic extracts of *Saponaria vaccaria* seed derived from several varieties, as well as various purified components obtained through successive chromatographic separations of total extracts were evaluated for their growth inhibitory activity in WiDr (colon), MDA-MB-231 (breast), NCI-417 (lung) and PC-3 (prostate) human cancer cells as well as the non-tumorigenic fibroblast BJ (CRL-2522) cell line using MTT colorimetric assay. Purified bisdesmosidic saponins segetoside H and I were further examined using microscopy and apoptosis assays. Bisdesmosidic saponins exhibited dose-dependent growth inhibitory and selective apoptosis-inducing activity. Growth inhibitory effects were particularly strong in a breast (MDA-MB-231) and a prostate (PC-3) cancer cell line. Total extracts exhibited a different preference being most active against a colon cancer cell line (WiDr). In a comparison of varieties, all of the total seed extracts exhibited similar dose-dependent activities, but with some variation in potency. Monodesmosidic saponins vaccarosides A and B, phenolic vaccarin, and cyclopeptide segetalin A, co-occurring seed substituents, did not exhibit activity. The non-tumorigenic fibroblast cell line BJ (CRL 2522) was growth inhibited but did not undergo apoptosis when treated with bisdesmosidic saponins at low micromolar concentrations. Saponin-rich extracts from *Kochia scoparia* seed and *Chenopodium quinoa* were also evaluated alongside *Saponaria* saponins but did not exhibit activity. Closely related Quillaja saponins exhibited activity but were less potent.

1. Introduction

The seed of the medicinal plant *Saponaria vaccaria* L.,[1] is rich in flavonoids, cyclopeptides and significant amounts of mono- and bisdesmosidic triterpene saponins (see Fig. 1) [1,2]. It has been used in traditional Chinese medicine (Wang Bu Liu Xing) for promoting menstrual discharge and milk secretion, healing wounds, as a diuretic, astringent, and anti-cancer agent [3–5]. Saponin-rich plants have been used since ancient times for the treatment of a variety of ailments with saponins possessing structure-dependent biological and pharmacological properties at least partly responsible for the inherent activities, e.g. hemolytic, antifungal, anticholesterolemic, anti-inflammatory, immunostimulatory, anti-cancer, etc. [6–9]. Cyclopeptides and flavonoids, the other classes of compounds present in significant quantities in the seed, have also been noted in several medicinal species and also exhibit a wide range of structure-dependent bioactivities [10,11].

Abbreviations: IC_{50}, 50% inhibitory concentration;MS, mass spectrometry;MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PAD, photodiode array detection.

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[1] A.k.a. *Vaccaria segetalis, V. hispanica, V. pyramidata;* com. cow cockle, cow herb.

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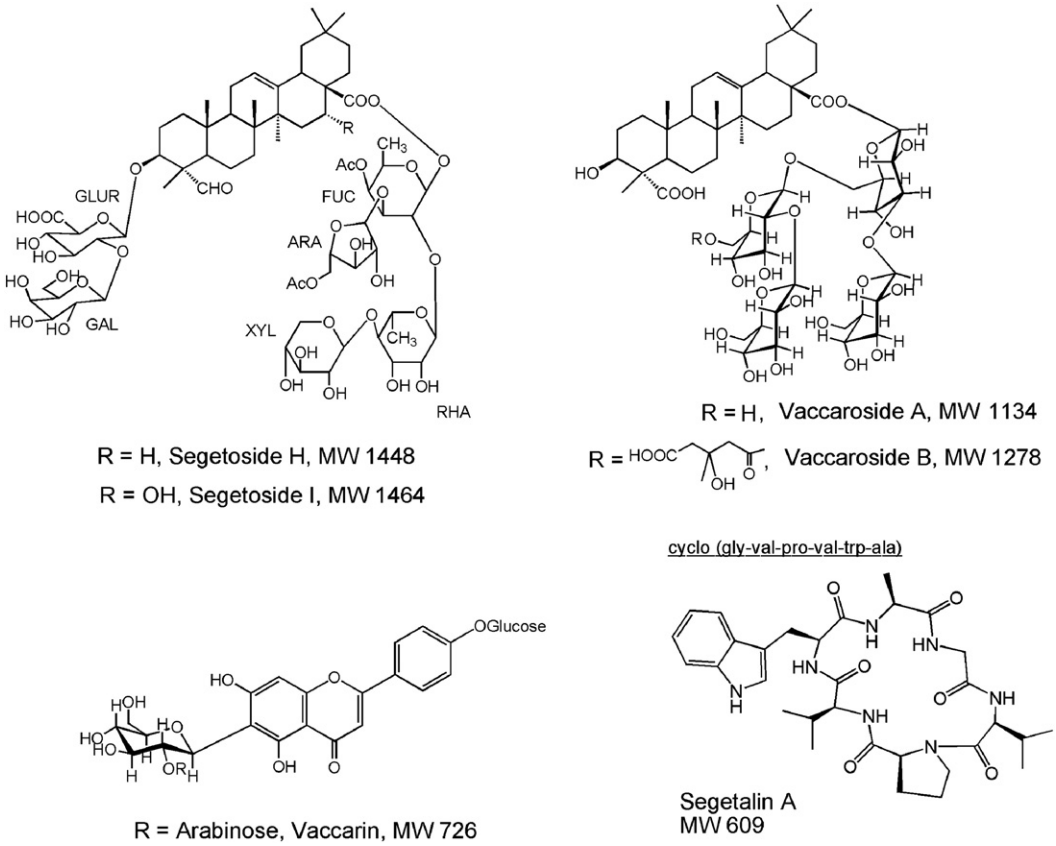

Fig. 1. Structures of some *S. vaccaria* seed constituents.

Seed extracts of this plant have shown potential anti-cancer activity. A screening study on the cytotoxic activity of aqueous extracts derived from seventy-one Chinese medicinal herbs conducted previously, indicated that the water extract of *S. vaccaria* inhibited the growth of a number of human and murine cancer cell lines (breast, lung, pancreas and prostate) *in vitro*[12,13]. More recently several purified saponins from *S. vaccaria* were reported to possess growth inhibitory activity against murine leukemia P388, human lung A549, and human prostate LNCaP cell lines *in vitro* [14]. Earlier, undefined fractionated seed extracts were observed to extend the life of tumor-implanted mice [15] and to reduce the volume of implanted tumors [16].

As much of the previously reported work was somewhat lacking in detail, it was decided to further investigate the antiproliferative activity of *Saponaria vaccaria* by comparing activity of seed extracts from different varieties, as well as other saponin-rich species, against a panel of human cancer cell lines, and to also further examine individual compounds found in the seed to ascertain the main sources of the reported antiproliferative properties and their possible mode of action.

2. Materials and methods

2.1. Plant material and seed extracts

Different varieties of *S. vaccaria* seed were obtained for this study. *S. vaccaria* 'Scott WT' (wild type, SWT) was obtained from Eric Johnson, Agri-Food and Agriculture Canada, Scott Experimental Farm, Scott, Saskatchewan. Seed of *S. vaccaria* cv. 'Pink Beauty' (PB) and 'White Beauty' (WB) were obtained from CN Seeds Ltd, Pymoor, UK, and the Mongolia variety (MG) was obtained from the North Central Regional Plant Introduction Station, USDA-ARS, Ames, IA, USA (accession PI 597629 originating from Mongolia). Several rows of each seed variety were hand seeded in the University of Saskatchewan Horticulture plots in the summer of 2003 and 2004. Seeds were planted 1 to 2 cm. deep at a rate of 100 seeds per row (7 m). Plants were grown under dry land conditions except for initial watering to promote germination. Bulk seed was harvested in the fall. *Quillaja* saponin having a sapogenin content of 25% (ca. 100% saponin content) was purchased from Sigma (S4521), Oakville, Ont. Canada. *Quinoa* saponin was obtained from HeadsUp Plant Protectants Inc., Kamsack, SK, Canada. *Kochia* saponin was obtained from processing *Kochia scoparia* seed as described in Ref. [17] Wang Bu liu Xing powdered seed was obtained from Botanicum Herbs, Box 329, Pembina, ND, USA.

2.2. Comparison of seed bioactivity from different Saponaria varieties

Ground seed (1 g) of each variety was mixed with 70% methanol (10 mL) and let stand overnight. The mixture was centrifuged and the pellet washed with a further portion of 70% methanol. The combined methanolic extract was