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*NOTE*

# An Anticancer Tannin and Other Phenolics from *Limonium axaillare* (Fam. Plumbaginaceae)

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Flavonoids, phenolics, coumarins and condensed tannins were isolated and identified from the leaves of *Limonium axaillare*. This is the first report of an ellagitannin, geraniin, from this genus. The anticancer activity of geraniin was found to be slightly higher than that of the crude extract.

The Plumbaginaceae is a family of two tribes, the Plumbagineae and the Staticeae (e.g., *Limonium*). Leaves and petals of *Limonium* species are rich in myrecetin, quercetin, and kaempferol glycosides[1–3]. The tannins of some *Limonium* species are of the condensed leucoanthocyanidin type[4].

We now report from *Limonium axaillare* Bark (Gold Dust), the isolation and identification of six coumarins, namely, xanthotoxol, imperatorin, aesculetin, isoimperatorin, bergaptin and umbelliferone and four known flavonoids, quercetin 3-O-rhamnoside, myrecetin 3-O-glucoside, quercetin and kaempferol, the known phenolics: ferulic, isoferulic, gallic and ellagic acids, and geraniin, a hydrolyzable tannin having a hexahydroxydiphenoyl (HHDP) group, galloyl group and dehydrohexahydroxydiphenoyl (DHHDP) group. This is the first report of an ellagitannin in this tribe having the latter type of group. The compound was found to have a moderate inhibition of *Ehrlish ascita* carcenoma cells. We previously reported another tannin anticancer compound containing HHDP and gallagyl groups (the latter is a tetramer of gallic acid) from *Terminalia arjuna*[5].

## Plant material

The bark of the plant *Limonium axillare* was collected in December 1997 from Halaieb at the Red Sea coast (a voucher specimen is deposited in the NRC Herbarium, Cairo, Egypt). The material was dried, powdered, and extracted with 80% aqueous acetone, and the extract was concentrated under reduced pressure. The concentrated material was fractionated over a Sephadex LH-20 column using ethanol, then ethanol-water mixtures for gradient elution to yield eight fractions. Each fraction was further analysed using preparative paper chromatography using

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either BAW or 15% aqueous acetic acid. Further purification employed Sephadex LH-20. The phenolics were identified by co-chromatography with authentic samples and color reactions. The structures were confirmed by $^{1}H$- and $^{13}C$-NMR spectroscopy[3, 6, 7].

**Anticancer studies**

Female Swiss albino mice weighing 18–20 g obtained from the breeding unit of the National Research Center, Cairo, Egypt, were used in this study. *Ehrlish ascita* carcinoma cells were supplied through Dr. C. Benckukh, Netherlands Cancer Institute, Holland.

The tumour line was maintained in female mice by weakly intraperitoneol transplanation of $2.5 \times 10^6$ cells. The ascitic fluid was diluted with normal saline so that each 0.2 mL contained $2.5 \times 10^6$ cells. The cells were counted microscopically using a haemacytometer, and the mice were inoculated with the tumor cells in a concentration of $2.5 \times 10^6$ cells/mouse. After 1 h of tumor inoculation, the mice were divided into eleven groups of 10 mice each.

**Treatments**

The group of ascitic mice were given injections of 0.2 mL using different concentrations of the tested extract and of geraniin to study their growth inhibition effect. One group served as control. After 24 h of inoculation all animals were weighed daily.

**Biological activity**

Results of the *in vivo* antitumour activity of the compound and the aqueous acetone extract against carcinoma cells in mice are as follows:

| Sample no | MST ± S.D. | T/C (%) |
|---|---|---|
| Control | 10 ± 0.8 | 100 |
| *L. axillare* extract | 23 ± 0.7 | 230 |
| Geraniin | 25 ± 0.8 | 250 |

MST : mean survival time

S.D. : Standard deviation

T/C : MST (Standard)/MST (control) × 100

Geraniin and the aqueous acetone extract increased the mean survival time of the mice by 25 and 23 days, respectively.

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