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Isolation, Cytotoxicity Evaluation and HPLC-Quantification of the Chemical Constituents from *Prangos pabularia*

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Abstract

Phytochemical analysis of the dichloromethane:methanol (1:1) extract of root parts of *Prangos pabularia* led to the isolation of twelve cytotoxic constituents, viz., 6-hydroxycoumarin (**1**), 7-hydroxycoumarin (**2**), heraclenol-glycoside (**3**), xanthotoxol (**4**), heraclenol (**5**), oxypeucedanin hydrate (**6**), 8-((3,3-dimethyloxiran-2-yl)methyl)-7-methoxy-2H-chromen-2-one (**7**), oxypeucedanin hydrate monoacetate (**8**), xanthotoxin (**9**), 4-((2-hydroxy-3-methylbut-3-en-1-yl)oxy)-7H-furo[3,2-g]chromen-7-one (**10**), imperatorin (**11**) and osthol (**12**). The isolates were identified using spectral techniques in the light of literature. 3-(4,5-dimethyl thiazol-2yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity screening of the isolated constituents was carried out against six human cancer cell lines including lung (A549 and NCI-H322), epidermoid carcinoma (A431), melanoma (A375), prostate (PC-3) and Colon (HCT-116) cell lines. Osthol (**12**) exhibited the highest cytotoxicity with IC_{50} values of 3.2, 6.2, 10.9, 14.5, 24.8, and 30.2 μM against epidermoid carcinoma (A431), melanoma (A375), lung (NCI-H322), lung (A549), prostate (PC-3) and colon (HCT-116) cell lines respectively. Epidermoid carcinoma cell line A431 was sensitive to most of the compounds followed by lung (A549) cancer cell line. Finally a simple and reliable HPLC method was developed (RP-HPLC-DAD) and validated for the simultaneous quantification of these cytotoxic constituents in *Prangos pabularia*. The extract was analyzed using a reversed-phase Agilent ZORBAX eclipse plus column C_{18} (4.6×250 mm, 5 μm) at 250 nm wavelength using a gradient water-methanol solvent system at a flow rate of 0.8 ml/min. The RP-HPLC method is validated in terms of recovery, linearity, accuracy and precision (intra and inter-day validation). This method, because of shorter analysis time, makes it valuable for the commercial quality control of *Prangos pabularia* extracts and its future pharmaceutical preparations.

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Introduction

Prangos, one of the largest and most widely distributed genus of family Umbelliferae consists of around 30–40 species. Most of the *Prangos* species are reported to possess diverse pharmacological activities viz. anti-microbial [1], anti-oxidant [2], cytotoxic [3,4], anti-helmintic and aphrodisiac [5], besides, their use in the treatment of haemorrhoids, wounds and leukoplakia [6]. In Central Asia, the extracts of *Prangos* species have been used to stop bleeding and heal scars [4].

Prangos pabularia commonly known as "Komal" in Hindi and "Kurangas" locally (Kashmir), occurs in stony slopes of Ladakh (Jammu and Kashmir, India). It is the only species of the genus found in India. In Indian traditional system of medicine, its roots and fruits have been used as diuretic, carmative, laxative, stimulant and liver tonic [7]. It is also used for treatment of itches, and as a promoter for the expulsion of foetus [7]. An infusion of the roots is useful in indigestion, flatulence and regularization of menstrual cycle in females [8].

Previous phytochemical investigations on the fruits and roots of *p. pabularia* revealed the presence of various chemical constituents, consisting of coumarins of diversified structures, terpenoids and glycosides [9–11]. It may be pertinent to say that coumarins form an important class of compounds known to possess various pharmacological activities viz. anti-inflammatory, anti-pyretic [12], anti-oxidant [13], bronchodilator [14], vasodilator [15], anti-amoebic [16], anti-bacterial [17] and anti-fungal [18]. Physiological, bacteriostatic and antitumor activity make these compounds attractive for further backbone derivatization and screening as novel therapeutic agent/s [19]. Weber and co-workers have shown that coumarin and its metabolite 7-hydroxycoumarin exhibit antitumor activity against several human tumour cell lines. In addition, it has been shown that