HPLC Quantification and Evaluation of Anticancer Potential of Bioactive fractions and crude extract of medicinal plant *Saussurea lappa* (Falc.) Lipsch.

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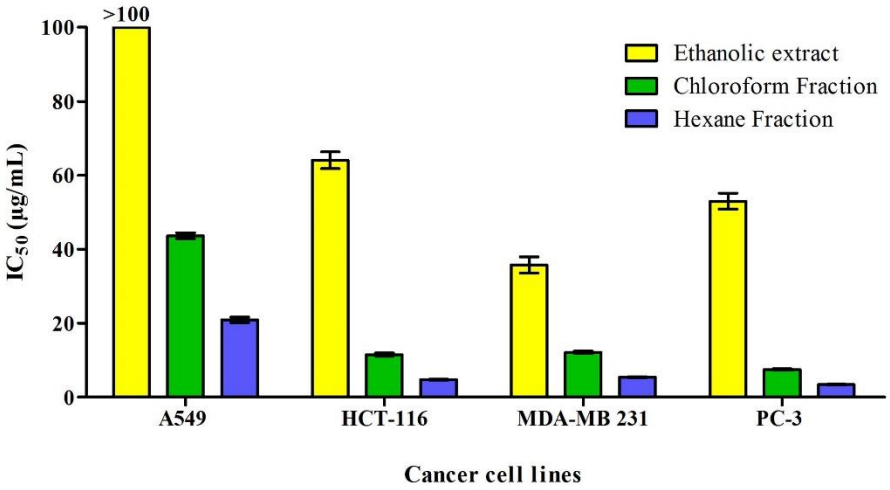
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Abstract: *Saussurea lappa* (Falc.) Lipsch (a synonym of *Aucklandia costus* Falc.) a perennial herb belonging to family Asteraceae is found abundantly in Himalayan region of China, India and Tibet. The rhizome is an important herb in traditional system of medicine due to its biological properties such as hepatoprotective, antiulcer, antimicrobial, antiparasitic, antioxidant, anti-inflammatory and has an exceptionally high anticancerous activity. The objective of our study was isolation and quantification of four standard compounds in crude extract and different fractions of *S. costus* and evaluation of the anticancer activity. The dried rhizome was powdered and extracted with 70% ethanol and fractionated using solvents of varying polarity such as hexane, chloroform and butanol. The four compounds Dehydrocostus lactone, Costunolide, Syringin and 5-Hydroxymethyl-2-

furaldehyde were isolated from different fractions and were used as standard compounds for quantification. The standardization of crude extract and bioactive fractions was carried out on a Thermo fischer HPLC system having software chromeleon (version 7.2.9). The retention time of chromatographic peak was obtained at 23.55, 22.84, 10.85 and 9.56 min for Dehydrocostus lactone, Costunolide, Syringin and 5-Hydroxymethyl-2-furaldehyde respectively. A reverse phase high performance liquid chromatography (RP-HPLC) method has been developed and validated. The method developed for separation and quantification consist of mobile phase having 0.1% formic acid in water as solvent A and methanol as solvent B. The chromatographic separation was carried out using lithosphere RP 18 end capped column (250 mm×4 mm, 5µm) keeping temperature at 35 °C. The solvent flow was optimised at 0.8 ml/min and injection volume was kept 10 µL. The chromatographic data showed good resolution and excellent linearity ($r^2 > 0.993$). The validation parameters such as inter and intraday precisions (RSD < 1.96%) and analyte recoveries (97.52-110.20%; RSD < 2.00%) depicted high sensitivity and reliability of the developed HPLC. The results confirmed that Dehydrocostus lactone and Costunolide were concentrated in hexane (222.08 and 65.07 µg/mg respectively) and chloroform fractions (99.02 and 30.21 µg/mg respectively) while butanol fraction majorly contains Syringin and 5-Hydroxymethyl-2-furaldehyde (37.91 and 7.94 µg/mg respectively). Further the SRB assay was performed for fractions and crude extract using lung, colon, breast and prostate cancer cell lines. The hexane and chloroform fraction show excellent IC_{50} value of 3.37 ± 0.14 and 7.527 ± 0.18 µg/ml respectively, against prostate cancer cell line (PC-3). The results confirmed that *S. lappa* could be a useful source of anticancer natural products and a validated method was essential for quality control of *S. lappa*.



Keywords: *Saussurea lappa*; RP-HPLC; isolation; quantification; dehydrocostus lactone; costunolide; syringin; 5-hydroxymethyl-2-furaldehyde, SRB assay

Publications:

1. **Bhushan, Anil;** Rani, D.; Lone, B.A.; Tabassum, M.; Kumar, S.; Gupta, P. N.; Gairola, S.; Gupta, A. P.; Gupta, P. "HPLC-PDA Method for Quantification of Bioactive Compounds in Crude Extract and Fractions of *Aucklandia costus* Falc. and Cytotoxicity Studies against Cancer Cells". *Molecules* **2023**, *28*, 4815. doi.org/10.3390/molecules28124815.
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I worked independently on the isolation and synthesis of semisynthetic analogues and gained expertise in multistep organic synthesis. During my doctoral training, I focused mainly on natural product-driven drug discovery by designing and synthesizing small bioactive molecule. Through meticulous bench work I gained good command in isolation of natural metabolites and multistep organic synthesis and acquired competence in the design, synthesis of novel therapeutic agents and structural characterization techniques (NMR, Mass spec, LC-MS, HPLC, UV, IR).