

^a Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy;

^b Department of Precision Medicine, University of Campania Luigi Vanvitelli, Vico L. de Crecchio 7, 80138 Naples, Italy; ^c Department of Science, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy; ^d Institute of Biosciences and Bioresources, Consiglio Nazionale delle Ricerche (IBBR-CNR); ^e Department of Pharmacy, University of Naples Federico II, Via Pansini, 80131 Naples, Italy

pasquale.marino@unibas.it

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Nuove Prospettive in Chimica Farmaceutica

Introduction

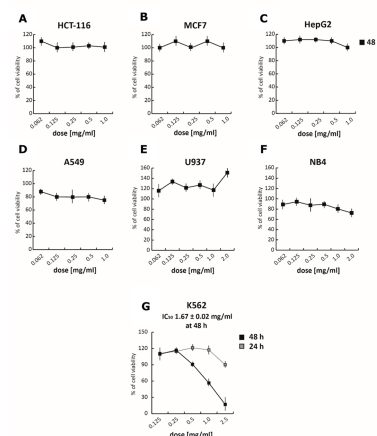
Chronic myelogenous leukemia (CML) is one of the most common tumors. CML is caused by a genetic alteration of the Philadelphia (Ph) chromosome, which forms the hybrid protein BCR/Abl¹.

Inhibiting BCR-ABL expression has long been the gold-standard approach in CML treatment.

Trifolium repens (TR), commonly known as white clover is used as a fodder crop for cattle, but to date, its potential anticancer activity has not been explored². In this study, we investigated the antitumor action of TR in several cancer cell lines, focusing specifically on its effect in CML cells.

In the CML K562 cells (Fig. G), the TR total extract reduced cell proliferation by about 50% and 80%, at 0.5 mg/mL and 1 mg/mL concentrations.

TR showed good cytotoxicity in K562 cells, with an IC₅₀ value of 1.67 mg/mL at 48 h of treatment (Fig. G).

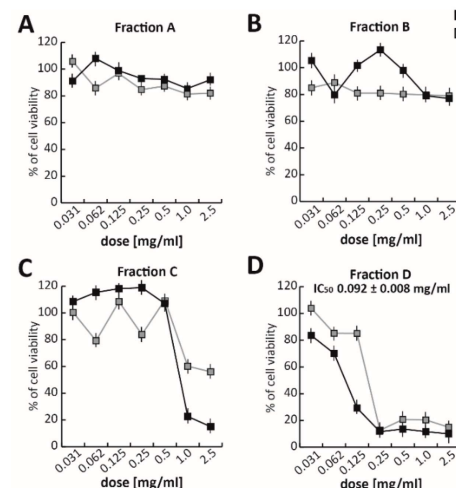


Results



To identify the fraction responsible for its anticancer activity, TR total extract was fractionated into four different fractions (A-D), based on their hydrophobicity and, thus, elution times (about 5 min for each fraction).

Fraction D induced a strong proliferative arrest in K562 cells at 0.25 mg/mL, at both 24 and 48 h. At 48 h, Fraction D showed an anticancer activity 10-fold greater than the total extract with an IC₅₀ value of 0.092 mg/mL (Fig. D).



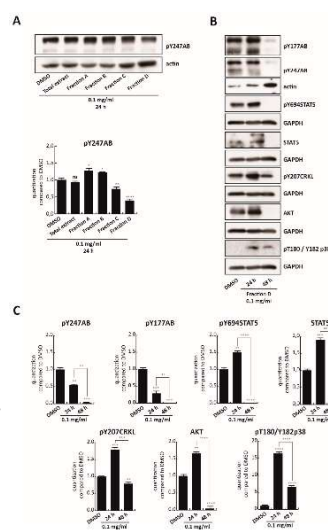
To confirm the role of Fraction D in inhibiting BCR/Abl, K562 cells were treated with all four fractions as well as with the total polyphenol extract at 0.1 mg/mL for 24 h.

Only Fraction D reduced the anti-pY247AB expression levels (Fig. A).

Fraction D totally abolished constitutive phosphorylation of BCR on Y177 as well as in Y247 (Fig. B, C).

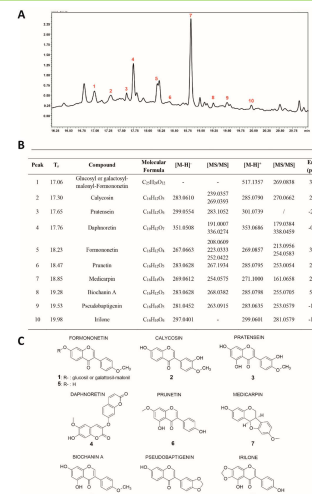
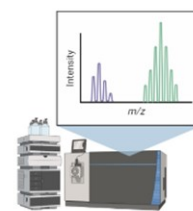
The total inactivation of BCR/Abl at 48 h was associated with abrogation of anti-pY694STAT5 and its total levels, as well as reduced levels of both anti-pY207CRKL and AKT.

In contrast, Fraction D promoted p38 expression >16-fold after 24 h and >7-fold after 48 h, compared to the control.



Fraction D was characterized by RP-UHPLC-MS/MS (Fig. A) and nine compounds were identified (Fig. B, C).

Among the isoflavones identified, the most abundant aglycone was found to be Medicarpin.



Conclusion

In this study, we found for the first time that TR exerts antitumor effects in CML. These biological effects are mediated by its isoflavonoid-rich portion, Fraction D. This fraction displayed low toxicity in normal cells, potentially making it an excellent option for chemotherapy. Our preliminary findings suggest that the development and biochemical optimization of phytochemical molecules contained in *T. repens* might lead to the identification of therapeutic agents active against CML.