Sesseli tomentosum Vis. is a species belonging to the Apiaceae family, whose members have been used in traditional medicine since ancient times due to the significant content of biologically active secondary metabolites. It is a species endemic to Croatia, Bosnia and Herzegovina, widely distributed in the coastal areas of the Dinarides. We present the first report on the chemical analysis of the volatiles of S. tomentosum. Headspace solid-phase microextraction (HS-SPME) followed by GC-MS analysis identified the following constituents as the most abundant in the fresh plant material: β -caryophyllene (13.58%), α -copaene (9.78%), germacrene D (6.99%), tetradecanal (6.89%), germacrene D (6.99%), δ-cadinene (5.29%), and dodecan-1-ol (4.29%). The chemical composition of the volatiles and the complete lack of data on biological activities of S. tomentosum extracts led us to investigate some of its potential biological activities. We investigated the antiphytoviral activity of S. tomentosum extracts against tobacco mosaic virus (TMV) infection and its cytotoxic activity in one healthy and three cancer cell lines. Plants of Nicotiana tabacum L. treated with hydrosol showed a significant reduction in virus concentration (50.05%) compared to control plants. The results indicate that the hydrosol of S. tomentosum, as an environmentally friendly, nontoxic byproduct of water distillation, is rich in biologically active constituents with significant antiphytoviral potential. The methanolic extract of S. tomentosum showed a significant cytotoxic effect on osteosarcoma (U2OS) and cervical adenocarcinoma (HeLa) cell lines with an IC₅₀ values of 452.305 µg/mL and 294.93 µg/mL, while it showed a weaker effect on colon cancer (HCT116) and human retinal pigment epithelial-1 (RPE1) cell lines with IC₅₀ values of 892.65 µg/mL and 772.41 µg/mL, respectively. Research on this valuable and unexplored species, its various extracts and individual biologically active compounds needs to be continued to fully explore its potential for use in the cosmetic, food and medical industries.