High consumption of fruits and vegetables is associated with a reduced risk of cancer. Polyphenolic compounds play a central role in this beneficial effect. Wine industry produces large amounts of residue rich in bioactive polyphenols from grape. In Brazil, the majority of grape by-products is discarded. Therefore, studies on the chemical composition of grape residue and on the investigation of its potential bioactivity are crucial to reduce environmental impact and to add value to the grape. The purpose of this study was to evaluate the cytotoxicity of an extract produced from grape pomace, a residue composed of skin and seed, in human breast cancer cells MDA-MB-231. Grape pomace was provided by Embrapa Uva e Vinho/RS, Brazil. Hydroalcoholic extract was prepared and concentrated by nanofiltration. Grape Pomace Extract (GPE) was characterized regarding its polyphenol content and anthocyanins profile by high-performance liquid chromatography. Biological activity was assessed after MDA-MB-231 incubation with 0.085 to 1.4 mg / mL (dry matter/mL media) of GPE up to 24 h. Viability assays and optical microscopy were used to investigate GPE cytotoxicity. GPE presented high content of polyphenols. Anthocyanins are the predominant class of polyphenols, in which cyanidin-3-O-glucoside, delphinidin and cyanidin-3-O-rutinoside correspond to 0.1, 0.14 and 6.3 % (g / 100 g dry matter), respectively. GPE was toxic to MDA-MB-231 cells in a concentration and time-dependent manner. A maximum of 30% decrease in cell viability was observed after 24 h incubation with 0.34 mg / mL of GPE, which corresponded to 20 µg cyanidin-3-O-rutinoside. The morphological changes induced by GPE suggest that apoptosis is involved in the cytotoxic effects. Biochemical mechanisms related to GPE cytotoxicity and apoptosis are under investigation. These results suggest the chemotherapeutic importance of bioactive polyphenols present in grape pomace from Brazilian wine industry and its nutraceutical potential.