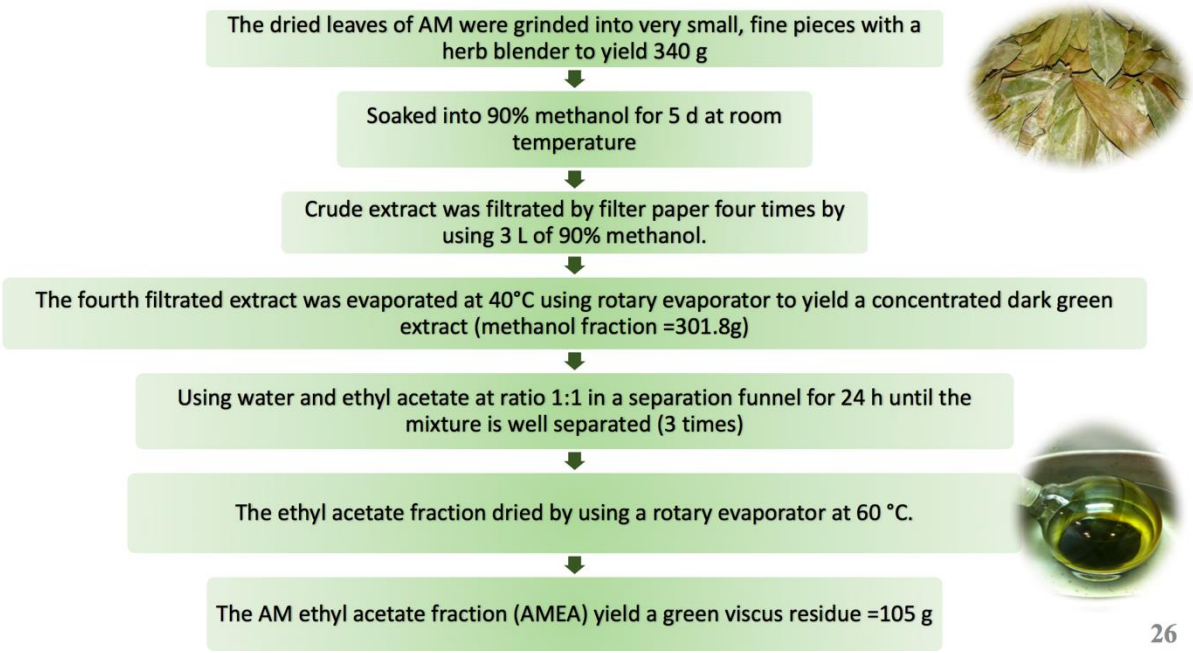
Supplementary materials

Schematic representation of the extraction of the compounds

Figure S1: Extraction of Annona muricata Ethyl Acetate



Basically, we used different methods that frequently used in plant extractions including plant tissue homogenization procedure and perculation with some modifications as following:

The dried leaves of AM were grinded into very small, fine pieces with an herb blender to yield 340 g. These grounded leaves were then soaked into 90% methanol for 5 days at room temperature. Then, this crude extract was filtered by filter paper four times by using 3 L of 90% methanol. The filtrated extract was then pooled and evaporated at 40°C using Buchi Rotavapor (Gaithersburg,MD) to yield a concentrated dark green extract. This methanol fraction was then subjected to a partition between water and ethyl acetate at ratio 1:1 in a separation funnel for 24 h or until the mixture was well separated; this step was repeated three times. After that, the ethyl acetate fraction was dried by using a rotary evaporator at 60 °C. The ethyl acetate fraction was thick green liquid residue and the final weight obtained was 105 g. This is the final AMEA extract which was used for further experiments.

Preparation of the fractions

The ethyl acetate extract was subjected to a preparative thin layer chromatography (TLC) plate (EMD Millipore, Billerica, MA) eluted by 90% ethyl acetate and 10% hexane. From this preparative TLC plate, eight individual bands were collected as shown in Figure S2. Each band was scraped and removed from the plate and soaked in ethyl acetate for 1 day. After that, each fraction was filtered by filter paper into evaporating flask in order to be evaporated by rotary evaporator at 40 °C. All the eight fractions were then tested on the BT-20 triple negative breast cancer cell line by using MTS cell viability assay.

Figure S2: Preparative thin layer chromatography