Introduction:

The study of natural products for drug discovery is a vital field in pharmaceutical research. Over the past 25 years natural products have been the forefront of discovery in drugs, from aspirin to antibiotics. The purpose of this research project was to determine if a new natural product could readily kill a certain line of breast cancer cells. The natural product tested in the MTT assay of the MCF-7 breast cancer cells was the plant Juniperus virginiana. Juniperus virginiana is a gymnosperm indigenous to the eastern half of the United States as well as the eastern portion of Canada. For this study Juniperus virginiana was particularly useful because it is easily found in all counties in the state of Alabama. Samples of this species, more commonly called the Eastern Red Cedar, were taken from both the male and female portions of the plant to test.

Methods:

The male and female components of the Eastern Red Cedar (Juniperus virginiana) plant were collected to extract from first. The essential oils were extracted from both sexes and distilled. Twenty five grams of these oils were then combined with 100 grams of silica gel to be used in the separation. To separate both samples the technique of liquid chromatography was used. This method used a glass column packed with silica gel along with a liquid phase of distilled hexane. Once the column was tightly packed the sample from the male and female were added to their own respective column. The columns were ran at first with pure distilled hexane and then proportions of distilled hexane to ethyl acetate to move the more polar compounds through the column. Once hands appeared and moved to through the column fractions were collected. The fractions were given time for the solvent to evaporate off and the mass taken after. The mass of the fractions collected from the entire column was then compared to the mass of the original sample placed in the column to ensure the accuracy of the separation. After the masses were collected, the samples that had formed crystals were taken and recrystallized by the solvent diffusion technique. All the samples were then ran on TLC plates to check the purity of all the compounds collected. The compounds found with similar retardation factors on the TLC plates were then combined to form compiled fractions to test on the MCF-7 breast cancer cells.

Results:

The spectrophotometric data from the MTT assay of both the male and female whole extracts were successful in killing the MCF-7 cells. Once it was understood that the whole samples were killing they were separated in two liquid chromatography columns. In 73 extracts, from the female counterpart of the Juniperus virginiana, a total of 22.3323 grams was collected. Thin layer chromatography was performed and showed commonality between samples which reduced the extracts from 73 individual fractions to 19 combined fractions. From these combined extracts, combinations 8, 9, and 17 were then recrystallized. The male counterpart of the Juniperus virginiana gave a total of 14.0175 grams from 78 extracts. Once TLC was performed the 78 extracts were combined to give 11 different compounds. The extracts that were combined in samples 1, 2, and 8 were then recrystallized to increase the purity.

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Conclusions:

The MTT gave positive results to support the hypothesis that some compound in Juniperus virginiana is responsible in killing the breast cancer cells. The extracts collected from the female counterpart were very positive. The total used extract was 25.0 grams and the resulting fractions being 22.3323 grams gave indication that the separation used almost the entire sample. The recrystallization of the female sample was successful under the solvent diffusion method and have been prepared to apply to the cancer cells. Unfortunately on the male, portion of the Juniperus virginiana the results were not as ideal. The sample size originally separated was still 25.0 grams originally but the resulting 14.0175 gram was not a successful separation. The recrystallization from this sample was also unsuccessful. The samples did not form crystals under the solvent diffusion method and were therefore lost for testing.

Continuing Research:

The male sample of Juniperus virginiana is currently being separated for a second time by use of a liquid chromatography column to hopefully resolve the problems encountered in the first extraction. These samples will then be taken and recrystallized and evaluated by TLC. The extracted samples from both the male and female separations will then be applied to the MCF-7 cells in a MTT assay to determine which fraction is specifically killing the cells. Once this has been determined a C13 NMR spectrum and an H NMR spectrum will be ran on the sample(s), to determine the exact structure. This starting research has given a perfect platform for this author’s continuing senior research in the coming scholastic year.

Reference: