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Anti-Cancer and Anti-Microbial Activity of Selected Hawaiian and Sonoran Desert Plant Species

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Background

Previous research into the anti-microbial and anti-cancer properties of certain plant species provided the basis of this project. Plants from various parts of Hawaii and the Sonoran Desert in Arizona had been obtained through interviewing of traditional medicinal practitioners in those areas. The plants were then screened at BYU for any significant inhibition of cancer and/or microbial growth. Significant inhibition was shown by any plant that inhibited cancer tissue or microbial growth by more than 50% over a 24 hour growing period. From the original stock of plants, 20 exhibited significant growth inhibition against one or more of the selected cancer or microbe lines. These plants were the focus of this ORCA supported study.

Review of Experimental Design

The purpose of this study was to further screen the 20 selected plants species for significant activity and to prepare between 4 and 7 of the most effective samples for further study by the National Institute of Health's Natural Products Division. The tests were conducted between October 2002 and April 2003 at BYU under the supervision of Dr. Rex Cates.

The first step in the secondary screening was to narrow down the number of organic plant compounds being looked at in each sample. The fractions obtained in the initial screening had been separated by chemical means into two groups of compounds: polar and nonpolar. In each case either one or the other of these fractions was more active. The secondary screening involved taking the active fraction (polar or nonpolar) and using chemical means to further divide the extract into two more fractions, each containing a smaller number of compounds. The purpose in further fractionating the plant extracts was twofold: to magnify any inhibitory activity previously expressed, and to facilitate any further research by narrowing down the number of compounds being looked at, the goal being to eventually isolate the active organic molecule(s).

Once the fractionations were completed the plant extracts were tested against the organisms that they had previously been shown to inhibit. Extract fractions were diluted to concentrations of 12.5, 25, 50, 100, and 200 $\mu\text{g/ml}$ and tested for activity against *Staphylococcus* (Staph infectious agent common in hospitals) and *Candida* (yeast infectious agent). Extracts were also tested at 6.125, 12.5, 25, 50, and 100 $\mu\text{g/ml}$ against a cervical cancer tissue line (HeLa) ; along with this cancerous line the extracts were tested against a non-cancerous tissue line (3T3) that was used to ensure that the extract was

selective for the cancerous cells. The diversity of concentrations was used to give an idea of the manner in which inhibitory activity decreases with respect to concentration.

Results, Analysis and Conclusion

In choosing the plants to be selected for further study, a growth inhibition of greater than 50 % in the highest concentration of extract was desired against the microbial and cancerous tissue lines. An inhibition of less than 25 % against the non-cancerous tissue line was also desired in the case of those extracts showing a high inhibition against the cancerous line. The results of the tests are shown in the Appendix; The (I) and (S) categories signify the separate extract fractions for each plant.

Four plants were shown to meet the above criteria: *Kalanchoe pinnata*, *Zinnia acerosa*, *Terminalia catappa*, and *Zauschneria latifolia*. Two additional plants were also noted as meriting further study based on their having inhibitory activity of above 40%: *Tribulus terrestris* and *Baileya multiradiata*. Out of the plants noted, three of them were highly active in both fractions, indicating that a better method of chemical separation could yield a better isolation. *Kalanchoe pinnata* exhibited a very interesting change in inhibitory activity against the cancerous tissue line as the concentration was decreased. Extract activity normally tends to decrease proportionally with the concentration of the extract; however, the activity of the *Kalanchoe* (S fraction) actually increased as the concentration dropped from 100 to 50 µg/ml. The activity then decreased slightly as the concentration was halved. The extract inhibited the cancerous growth by 72.6 % at a concentration of 100 µg/ml and inhibited growth by 77.4 % at 50 µg/ml. The inhibition then drops again to 71.4 % at 25 µg/ml. At the lowest concentration of 6.125 µg/ml, growth was still inhibited by 55.8 %. The non-cancerous inhibition was negligible below a concentration of 50 µg/ml.

Extract samples of the six plants mentioned were evaporated, and between 20 and 40 mg of each sample was set aside and packaged for delivery to the National Institute of Health. A literature search is being completed on each of the plants to determine the nature of any published research on these species, and will be included in the material to be sent with the extracts. Some work has already been started to determine the best way isolate and identify the individual active component(s) of each extract.