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Research Article

Cytotoxicity of Selected Medicinal and Nonmedicinal Plant Extracts to Microbial and Cervical Cancer Cells

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This study investigated the cytotoxicity of 55 species of plants. Each plant was rated as medicinal, or nonmedicinal based on the existing literature. About 79% of the medicinal plants showed some cytotoxicity, while 75% of the nonmedicinal plants showed bioactivity. It appears that Asteraceae, Labiatae, Pinaceae, and Chenopodiaceae were particularly active against human cervical cancer cells. Based on the literature, only three of the 55 plants have been significantly investigated for cytotoxicity. It is clear that there is much toxicological work yet to be done with both medicinal and nonmedicinal plants.

1. Introduction

There is a one-in-four chance that a drug used from any pharmacy has an active ingredient derived from a plant [1]. Indeed, the international consumer market for medicinal herbs and botanicals is estimated to be at about US $18 billion [2]. Hence, in our technological age, plants continue to play a significant role both medically and economically.

Even the most ancient written records of human civilization tell of humans using plants in everyday life. For centuries plants have been used to feed, clothe, and heal families. Examples of medicine that contains plant derivatives include aspirin, used for pain relief and inflammation reduction; physostigmine and pilocarpine, used for glaucoma control; quinidine, which has saved the lives of many heart attack victims.

The principal goal of this study was to determine if extracts from selected medicinal and nonmedicinal plants were cytotoxic; often, the difference between a therapeutic and a toxic extract or compound is simply the dose level. Our hope is that these survey data can be used as early indicators of some plants that may have therapeutic activity. Moerman has done extensive screening studies on a variety of medicinal plants [3]. From his investigation, we selected 55 plants representing 37 different species from 8 families. The four principal families, Asteraceae, Labiatae, Ranunculaceae, and Pinaceae, represent the first, third, fourth, and fifth families with the most medicinal species. It was hoped that our data might show some trends of toxicity within medicinally rich families.

The toxicity of each extract was determined in both prokaryotic and eukaryotic cells. Prokaryote cells included *Staphylococcus aureus,* a gram-positive cocci responsible for infections of the skin and respiratory tract, food poisoning, and toxic shock; *Salmonella choleraesuis,* a gram-negative facultative aerobe responsible for food poisoning; *Pseudomonas aeruginosa,* a gram-negative rod that causes infections in wounds. For the eukaryotic system, HeLa cells, an epithelial carcinoma of the cervix, were used.

2. Materials and Methods

2.1. Plant Extraction

(i) 50 g of plant tissue were collected and dried at 45°C.

(ii) The plant was ground in a Wiley Model no. 4 plant mill.

(iii) The ground material was then extracted in methanol for twenty-four hours.

(iv) The samples were filtered in glass-fiber filters fitted with coarse pore discs, and rotary evaporated down to 20 mL of extract on a Buchi RE111 Rotary Evaporator.

2.2. Microbial Bioassay

(i) Twenty-four hours before the assay, each of the three bacteria were grown in a culture tube with 5 mL of tryptic soy broth without dextrose and incubated at 35°C.

(ii) (14.5 cm) Petri dishes were previously prepared with a coat of Muller Hinton Medium (agar). The cultures were checked on a spectrophotometer to ensure the proper growth (20% transmittance at 600 nm). A lawn was then spread in the petri dish. Six 1.4 cm circles of filter paper were then coated in plant extract, three with 20 μL and three with 30 μL, and placed on the plate. A disk with 20 μL of water was added to the plate for a negative control and to S. *aureus,* S. *choleraesuis,* 10 μL of Ampicillin (BBL Sensi-Disc (Becton Dickinson)) was added as a positive control. The plates were incubated overnight at 35°C.

(iii) The plates were then collected the next day and the zones of inhibition were measured.

2.3. HeLa Assay

(i) HeLa cells were maintained and assayed in MEM with α modification (Sigma M-0894) supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 1x MEM-nonessential amino acids (Sigma M-7145), 2 mM L-glutamine, and gentamicin at 50 μg/mL.

(ii) Each extract was dried down and 2 mg/mL solutions were made using 10 mM Tris buffer at pH 7.4.

(iii) 150 μL of a solution of suspended HeLa cells diluted with 15 mL of α-MEM is added to each well of a 96 well plate and incubated overnight at 37°C and 5% CO_2.

(iv) The next day 75 μL, 50 μL, 25 μL with 25 μL of α-MEM, 12.5 μL with 37.5 μL of α-MEM, or 6 μL with 44 μL of α-MEM of the 2 mg/mL extracts was added to 9 wells as a control. The prepared plate was incubated overnight.

(v) The cells were arrested the next morning with 0.4 N perchloric acid. The perchloric acid is removed, and the cells were stained in 4% sulforhodamine B in 1% acetic acid and then washed in 1% acetic acid. The dye was allowed to dry and 150 μL of 10 mM Tris base unbuffered was then added to each well, and the absorbance of each well was read using a spectrophotometer at 570 nm.

(vi) The percent viability was calculated as the ratio of absorbance of the treated sample over the average of the controls. These values were then plotted and analyzed for a dose response.

3. Results

3.1. Microbial Assay. Of the 55 plants tested, only four, *Pinus monticola, Abies procera, Salvia vaseyi,* and *Salvia apiana*, inhibited the growth of S. *aureus.* The remaining microorganisms were unaffected by the extracts. However, the zones of inhibition were quite small, only about 1 cm each. The assay is rather a crude test when compared with the HeLa cell assay. This is understandable because the zone of inhibition is directly proportional to the concentration of the biologically active agent and its diffusibility, so the possibility of active compounds not showing a positive response could be expected if the active ingredients did not diffuse. Due to the screening nature of this procedure and small sample size, the quantitative analysis of the size of the rings of inhibition was quite subjective.

3.2. HeLa Cell Assay. The LC-50 s were calculated for each of the samples. Some of the extracts were so toxic to the HeLa cells that very low doses of 0.0l and 0.001 mg/mL were studied in order to establish an LC-50. The LC-50 s were calculated from least squares regression using the LINEST function on Microsoft Excel 2000 over the dose response range or the whole data set in the case of nontoxic extracts to get a rough quantitative value in order to assess cytotoxicity. Tris buffer, the control, gave an average 92% viability with no dose response. All values were adjusted up by 8% accordingly.

We experienced four general trends in the data. The first two types we labeled "A" for active. The first type was a clear dose-response over the full range of concentrations. Type two followed a steep dose-response over the initial range of concentrations while the lower concentrations did not. Type two was the most cytotoxic. Type three was labeled with an "M" for mildly active. These showed a weaker dose-response only at the higher concentrations. Type four was labeled "N" for not active. These samples showed no dose response and only marginal mortality. These trends were then evaluated over medicinal and family lines (Table 1).

4. Discussion and Conclusions

Of the 46 medicinal plant extracts, 54% were active, 26% were mildly active, and 20% were not active against HeLa cells. Thus, 80% of the medicinal plant extracts showed some type of cytotoxicity. This strongly suggests that there may be some connection between plants known from indigenous cultures to have medicinal properties compared to empirically determined cytotoxicity. Our eight non-medicinal plants also tended to be bioactive, with 50% active, 13% mildl, and 37% not active. Only four samples showed antibacterial activity, which was only in S. *aureus,* and all these extracts were from medicinal plants. Thus, only 14% of the medicinal plants showed limited antibiotic activity.

Asteraceae, the sunflower family and one with the highest medicinal activity rating in Moerman's paper [3], was the only family from which we had a fairly large sample, 15 medicinal plants. Extracts from Asteraceae tended to be quite active and followed the general trends of medicinal plant bioactivity as stated above with 54% active, 29% mildly active, and 17% not active. The mint family, Labiateae, also tended to be cytotoxic with 86% of the plants showing bioactivity. Because only seven plants were tested, more

Table 1: Cytotoxicity of selected plant extracts to bacterial cells and HeLa cancer cells.

Family	*Genus*	*Species*	Plant part	MD	BA	HA	LC 50 (mg/mL)	*m* (slope of the line)	*b* (*y*-intercept)	$\mathbf{r^2}$
Asteraceae	*Acanthospermum*	*australe*	Whole	Y	N	M	0.191	−38.817	57.414	0.112
Asteraceae	*Ambrosia*	*ambrosioides*	Areal	Y	N	M	−2.199	−13.656	19.970	0.469
Asteraceae	*Ambrosia*	*ambrosioides*	Leaf	Y	N	A	−2.446	−11.722	21.327	0.412
Asteraceae	*Ambrosia*	*ambrosioides*	Stem	Y	N	A	0.466	−152.941	121.230	0.857
Asteraceae	*Ambrosia*	*ambrosioides*	Root	Y	N	A	0.439	−124.376	104.617	0.817
Asteraceae	*Ambrosia*	*deltoidea*	Stem	Y	**	A	0.500	−83.064	91.544	0.293
Asteraceae	*Hieracium*	*caespitosum*	Whole	Y	N	M	0.669	−95.238	113.737	0.546
Asteraceae	*Anaphalis*	*margaritacea*	Whole	N	N	N	1.195	−51.360	111.373	0.530
Asteraceae	*Gutierrezia*	*microcephala*	Areal	Y	N	A	0.470	−143.776	117.641	0.969
Asteraceae	*Pyrrhopappus*	*carolinianus*	Whole	Y	N	N	0.117	−1052.510	173.100	0.623
Asteraceae	*Silphium*	*compositum*	S/L/Fl/R	Y	N	A	1.388	−49.271	118.400	0.134
Asteraceae	*Tetragonotheca*	*helianthoides*	Root	Y	N	A	0.436	−137.978	110.164	0.760
Asteraceae	*Tetragonotheca*	*helianthoides*	S/L/Fl/R	Y	N	M	0.357	−157.865	106.395	0.844
Asteraceae	*Erigeron*	*pumilus*	Whole	Y	N	A	0.366	−142.337	102.035	0.895
Asteraceae	*Liatris*	*secunda*	Whole	Y	N	M	0.531	−156.829	133.257	0.638
Asteraceae	*Cirsium*	*undulatum*	Areal	Y	N	M	0.884	−61.281	104.180	0.642
Asteraceae	*Thelesperma*	*filifolium*	Areal	Y	N	A	0.635	−99.503	113.172	0.950
Asteraceae	*Helianthus*	*nuttallii*	Stem	Y	N	A	0.539	−135.164	122.791	0.755
Asteraceae	*Helianthus*	*nuttallii*	Twig	Y	N	A	0.399	−181.986	122.686	0.944
Asteraceae	*Haplopappus*	*annuus*	Whole	Y	N	N	0.918	−77.726	121.359	0.803
Asteraceae	*Antennaria*	*parvifolia*	Whole	N	N	A	0.273	−81.056	72.160	0.497
Asteraceae	*Hymenopappus*	*filifolius*	Areal	Y	N	A	0.112	0.167	38.910	0.611
Asteraceae	*Centaurea*	*maculosa*	Twig/Fl	N	N	M	0.247	−113.161	77.895	0.863
Asteraceae	*Scorzonara*	*laciniata*	Root	N	N	N	1.838	−28.633	102.630	0.188
Boraginaceae	*Echium*	*candicans*	Stem	Y	N	M	0.394	−124.333	99.049	0.853
Chenopodiaceae	*Atriplex*	*confertifolia*	Areal	N	N	A	0.127	−1116.726	192.195	0.694
Chenopodiaceae	*Atriplex*	*confertifolia*	Rhizome	N	N	A	0.317	−142.791	95.259	0.838
Euphorbiaceae	*Bernardi*	*myicifolia*	Stem	N	N	A	0.488	−144.404	120.448	0.863
Labiatae	*Salvia*	*vaseyi*	Root	Y	N	A	0.105	−1109.581	166.468	0.981
Labiatae	*Salvia*	*vaseyi*	Stem	Y	N	A	0.399	−177.100	120.693	0.783
Labiatae	*Salvia*	*vaseyi*	Twig/L	Y	Y	A	0.218	−120.339	76.261	0.537
Labiatae	*Salvia*	*vaseyi*	Flowers	Y	N	A	0.231	−103.088	73.816	0.589
Labiatae	*Salvia*	*apiana*	Root	Y	Y	N	0.112	−1229.526	187.967	0.765
Labiatae	*Salvia*	*dorrii*	L/T/FloBu	Y	N	A	0.512	−125.827	114.434	0.852
Labiatae	*Lavandula*	*stoechas*	Root/Fl	Y	N	A	0.110	−1069.264	167.838	0.927
Labiatae	*Lavandula*	*stoechas*	Stem/L	Y	N	A	0.440	−196.726	136.532	0.804
Labiatae	*Lycopus*	*asper*	Stem	Y	N	A	1.043	−39.514	91.202	0.094
Labiatae	*Marrubium*	*vulgare*	Areal	Y	N	A	0.241	−112.266	77.093	0.768
Labiatae	*Satureja*	*douglasii*	Whole	Y	N	A	0.293	−102.001	79.891	0.665
Malvaceae	*Sphaeralcea*	*angustifolia*	Whole	N	N	A	0.304	−129.152	89.243	0.658
Pinaceae	*Pinus*	*monticola*	Bark/St	Y	Y	A	0.346	−175.851	110.896	0.764
Pinaceae	*Pinus*	*monticola*	Twig/L	Y	N	M	0.438	−78.452	84.370	0.396
Pinaceae	*Pinus*	*monticola*	Root	Y	N	A	0.573	−95.050	104.422	0.606
Pinaceae	*Picea*	*sitchensis*	Root	Y	N	N	0.496	−125.394	112.182	0.847
Pinaceae	*Picea*	*sitchensis*	Stem	Y	N	M	0.583	−118.928	119.326	0.562
Pinaceae	*Picea*	*sitchensis*	Bark	Y	N	M	0.366	−134.091	99.034	0.684
Pinaceae	*Picea*	*sitchensis*	Twig/L	Y	N	N	0.526	−121.778	114.038	0.835
Pinaceae	*Picea*	*sitchensis*	Cone	Y	N	A	0.438	−59.520	76.046	0.326
Pinaceae	*Abies*	*procera*	Root	N	Y	N	0.381	−155.741	109.290	0.978

Table 1: Continued.

Family	*Genus*	*Species*	Plant part	MD	BA	HA	LC 50 (mg/mL)	*m* (slope of the line)	*b* (*y*-intercept)	$\mathbf{r^2}$
Ranunculaceae	*Delphinium*	*geyeri*	Areal	Y	N	M	1.306	−29.140	88.057	0.173
Ranunculaceae	*Aquilegia*	*fromosa*	Root	Y	N	M	1.532	−25.856	89.605	0.222
Ranunculaceae	*Aquilegia*	*fromosa*	Flowers	Y	N	N	0.612	−82.675	100.574	0.534
Ranunculaceae	*Aquilegia*	*fromosa*	Leaf	Y	N	N	0.521	−143.711	124.894	0.784
Ranunculaceae	*Delphinium*	*glareosum*	Whole	Y	N	N	0.824	−91.704	125.548	0.428
Ranunculaceae	*Delphinium*	*nuttallianum*	Areal	Y	N	N	1.319	−35.840	97.287	0.350

MD: medicinal plant; Y: medicinal plant; N: non-medicinal plant
BA: bacterial assay; Y: inhibition; N: no inhibition; **sample lost
HA: HeLa cell assay; A: active; M: mildly active; N: not active
Plant part; S/St: stem Fl: flower; FloBu: flowering bush; A: areal R: root; L: leaf; T: twig.

data should be collected from this family before a general conclusion can be made about its cytotoxicity. Of the nine *Pinaceae* plant extracts, 67% showed some bioactivity. Additional work is needed to determine which plant parts tend to have the highest bioactivity. The least active of our five medicinal families was Ranunculaceae with two out of six plant extracts (33%) showing mild activity. Overall these data clearly suggest that non-medicinal as well as so-called medicinal plants should be used in general cytotoxicity screening evaluations. In fact, de Oliveira Maria et al. [4] also found significant bioactivity in 12 species of Amazonian plants which were non-medicinal.

Though this work proved to be insightful, future studies should be undertaken in order to get a clearer picture of the evolutionary relationship of bioactivity and medicinal ranking of plants. From the literature, it appears that only three plants from our group, *Ambrosia ambrosioides* [5, 6], *Gutierrezia microcephala* [7], and *Atriplex confertifolia* [8] have had extensive research on their cytotoxicity. Hence, there is a great deal of toxicology work yet to be done on the remainder of the plants shown to be bioactive in our investigation.

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