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The Cytotoxic Effect of Tropical Plants on Human and Rat Hepatoma Cells

Mark Tafazoli

The Cytotoxic Effect of Tropical Plants on Human and Rat Hepatama Cells

by
Mark Tafazoli

Honors Research Project
Spring 1993

THE UNIVERSITY OF ALABAMA IN HUNTSVILLE

Honors Program

HONORS SENIOR PROJECT APPROVAL FORM

(To be submitted by the student to the Honors Program with a copy of the Honors Project suitable for binding. All signatures must be obtained.)

Name of Candidate: Bahador Tafazoli

Department: Chemistry

Degree: Bio-chemistry

Full Title of Project: The Cytotoxicity Effect of Some Tropical plant Extracts on Human and Rat Hepatoma (Cancerous Liver) cells.

Approved by:

Debra M. Mairarity — 6/1/93
Project Advisor — Date

R. S. Campbell — 6/9/93
Department Chair — Date

Honors Program Director for Honors Council — Date

HPform.doc 05/07/93

Abstract

Compounds were extracted from over 60 tropical plants of Monteverde Cloud Forest Reserve in Costa Rica. A crude extract was obtained using 90% ethyl alcohol, which was later evaporated and extracts were redissolved in dimethyl sulfoxide (DMSO). Human and rat liver carcinoma cell lines, HepG2 and H4IIE cells, were maintained and the plant extracts were introduced to the cells. The viability of the cancer cells after exposure to the extracts was determined using an MTT assay. 8 plant extracts were found to kill over 90% of both the human and rat hepatoma cell cultures. These extracts were *Dendropanax quercettii, Oreopanax nubigenum, Oreopanax capitatus, Didymapanax pittieri, Oreopanax liebmannii, Orepanax oerstedianum* and *Dendropanax arboreus.*

Introduction

Medical botany is designed to bring into perspective the massive knowledge acquired by man to retain his health by using the plants around him. Man's survival has been dependent upon his innate curiosity, his desire to examine by trial and error all aspects of his environment, and to explore, for example, which materials are remedial, which ones are harmful, and which give him the greatest nourishment. This legacy exists today. Many of today's pharmacological drugs are based on natural products. For example, willow tree bark contains Salacylic acid, a derivative of which is aspirin. Ancient doctors asked their patients to chew on fresh willow tree branches in order to alleviate pain. *S. dulcamara*, a tropical plant, has been used to treat cancer, tumors, and warts from the time of Galen (A.D. 180); in addition, references to its use have appeared in the literature of many countries[1]. Taxol is a recently discovered compound isolated from the plant *Taxus brevifolia* which shows antitumor activity[2]. Such "historical remedies" have led to the discovery of many pharmacological drugs.

Due to advances in science and technology, today many of these drugs can be manufactured synthetically. With sophisticated techniques and devices, the active compound can be purified and the structures accurately determined. Additional research can inform the scientists about mechanisms or metabolic actions of the drugs. Many modern miracle drugs evolve in this manner.

The originally discovered natural product may then pave a way for discovering a new antitumor drug. Thus, much research and preparation follows the discovery of

such naturally produced drugs.

The objective of this research was to find and isolate natural organic compounds from tropical plants that have potential antitumor activity. Several criteria were used for the selection of tropical plants. The first being that several successful findings have been cited from plants in these tropical forests in the past[3]. Rain forests have humid climates that are suitable for bacterial and fungal growth, and many plants have to protect themselves against these microorganisms. As one measure of protection plants synthesize organic compounds that are either toxic or retard the growth rate of many of these rapidly growing organisms. Other fast growing cells such as tumor cells may also be retarded or killed upon exposure to some of these compounds. Since theses tropical forests contain plants that may have never been discovered, they make an even better candidate for research.

This project can be a part of the war against cancer. Once a plant extract is found to inhibit the growth of cancer cells, it can be further studied to find the chemical compound responsible for its effect. Organic synthesis of the compound and further structural modifications may also be performed to possibly optimize its effect. After further animal testing, pharmaceutical companies may in fact one day commercially industrialize a drug.

What is cancer? Cancer is a general term applied to all malignant tumors. As isolated tumor cells they creep into normal tissue space. Presumably, they do this by means of ameboid motion, a phenomenon that can be demonstrated in tissue culture. They squirm their way between the normal cells and may spread to great distances.

This tendency to filter into or invade tissue is spoken of as infiltration. Of even great danger to life is the capacity of malignant cells to enter the blood or lymphatic vessels. Once they pass through the thin wall of a capillary or liphatica channel, they can grow more easily. They fill the vessel and are often deadly. Cancer cells rapidly and uncontrollably multiply. Since the abnormal cells are no longer controlled by the genetic plan governing the orderly division of normal cells, they divide frequently in a disorderly fashion, often with varying chromosomal numbers. The cancerous cells mass together to form a growth or proliferate individually throughout the body. Either way they serve no useful function, but to invade. These growths may then interfere with the function of the organ or tissue in which they are found. They sometimes enter the bloodstream, to be carried to more distant parts of the body where other similar growths (metastases) are formed. Malignant cells also spread throughout the body through lymphatic vessels, which drain lymph from organs and tissues.

Cancer is not only the most feared of human maladies, it is also the most baffling. Man's recent research into the complex of several hundred different types of cancer has produced limited success. Forty years ago less than one-fifth of all known victims survived five years after diagnosis; now one-third live at least that long. Yet each year in the United States, there are more than 665,000 new cases of cancer and about 365,000 deaths from this disease. The direct medical expenses involved are estimated at $3 billion, but the agony to patients and the heartache for families and friends represent far more serious costs[4]. These scientific endeavors strive to discover a cure for this horrible disease.

Materials and Method

The preliminary screening of many of these extracted compounds was an essential part of the research. Compounds were extracted from over 60 tropical plants of Monteverde Cloud Forest Reserve in Costa Rica. A crude extract was obtained using 90% ethyl alcohol, which was later evaporated and extracts were redissolved in Dimethyl sulfoxide (DMSO). Human and rat liver carcinoma cell lines were maintained and the plant extracts were introduced to the cells.

A sterile environment was needed to grow the cell lines. Any exposure to contaminated equipment or reagents would invalidate any data gathered. Hence, meticulous care was taken to avoid such contaminations. All procedures were completed under a sterile hood with autoclaved equipment and sterile filtered reagents.

Two cell types were used in the experiment: HepG2, a human liver hepatoma cell line; and H4IIE, a rat liver carcinoma cell line. Each cell culture required separate culture protocols as shown.

Preparation of media:

The media mimics the cells' natural environment and includes the essential nutrients, vitamins & minerals, growth factors, and correct pH. Eagles Minimum Essential medium, EMEM, was the media used with HEPES buffer.

A bottle of EMEM was dissolved in approximately 850ml of sterile deionized water. 30ml of 1mM Hepes, 0.4ml of gentamicin (50mg/ml) were added to the solution under a sterile hood. The pH was adjusted to 7.35 with 10N NaOH, and the

volume was then brought to 1000ml by adding sterile deionized water. The solution was sterile filtered while splitting into 500ml autoclaved bottles using vacuum filtration.

H4IIE Media

For every 500ml media 62.5ml (10% v/v) of neonatal calf serum, and 62.5ml (10% v/v) of fetal calf serum were added asceptically.

HepG2 Media

a. Plating media

For every 500ml media 62.5ml (10% v/v) of horse serum was added asceptically.

b. Feeding Media

For every 500ml media 62.5ml (10% v/v) of horse serum, and 31.25ml (5% v/v) of bovine calf serum were added asceptically.

Preparation of cell lines:

Frozen cells were removed from liquid nitrogen and thawed in a 37°C water bath. Cells were plated in $25cm^2$,(T-25), flasks in concentrations of 5.0×10^5cells / flask with 5ml media (plating media for HepG2). Cells were incubated at 37°C, and media was changed every 48 hours, until cells were confluent and ready for passage.

Passaging Cells:

Cells were washed with 2ml of warm (37°C) Hanks Balanced Salt Solution, BSS. Two ml of trypsin:EDTA solution (1:10 dilution with Hanks BSS) was then added to cells and incubated at 37°C for 5 minutes. The released cells were then

pipetted into a sterile tube along with an equal volume of media to inhibit the trypsin activity. Cells were centrifuged (700xg, 5min) and resuspended in 5ml of media.

Cell Counting:

Cells were counted using a hemocytometer. 0.10ml of the re-suspended cells was added to 0.30ml trypan blue and 0.60ml 0.9% saline solution (1:10 dilution). Once the total number of cells was known, cells were then divided into cultures of 5×10^5 cells in T-25 flasks with 5ml media and 1.9×10^5 cells/well in 24-well plates with 1ml media. All cells were given fresh media every other day prior to assay.

Cytotoxicity assay:

Twenty five cm^2 flasks were used to maintain cell lines, whereas assays were performed in 24-well plates. 48 hours were allowed after plating to obtain a confluent layer of cells before assaying the compounds. Cells were fed with media (1ml/well) before addition of 25µl of extracts to each well. Each set of assay included a control with no compounds added, a DMSO control with 25µl of DMSO added, and a negative control with 25µl of 0.2M sodium azide added. The crude extracts dissolved in DMSO (0.1% w/v) were exposed to both hepatoma cell types and the cells were incubated for an additional 48 hours.

MTT Assay

After incubation 250µl of (3-(4,5-dimethylthiozol-2-yl)2,5-diphenyl tetrazolium

bromide), MTT, (2mg MTT/ml of PBS[1]) was added to each well. Following 4 hours of incubation, the media was carefully removed by aspiration and the blue crystals formed were dissolved in 1.0ml of ISO-PBS (100ml isopropanol, 50ml PBS, & 40µl 5N HCl) and allowed to sit for 5 minutes. The content of each well was then transferred to microfuge tubes and centrifuged for 3 minutes. The supernatant was then transferred to cuvettes and read on the spectrophotometer at 570nm against a ISO-PBS blank. The absorbance was recorded for each well, and duplicated runs were averaged.

Results

The absorbance for the repeated runs were averaged and the standard deviations were calculated. The percent viability against the DMSO control was calculated. The cytotoxicity against the hepatoma cells were determined from the percent viability against DMSO control - 100%. These results are shown in table 1. The extracts are assigned numbers for simplicity. Figures 1 and 2 illustrate the same data in graphical representation.

Conclusion

Figure 3 shows a healthy culture of HepG2 cells. Figure 4 shows a dead culture of HepG2 cells after exposure to extract 26, ***Dendropanax guercettii***. Figure 5 shows an unhealthy culture of HepG2 cells after exposure to extract 25, ***Oreopananx glabrous***. Figure 6 shows formazan crystals formed by healthy HepG2 cells after the

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[1] PBS-Phosphate buffered saline solution

MTT assay. Figure 7 is a higher magnification of these crystals. Figure 8 shows a low magnification of some internal crystals produced by unhealthy HepG2 cells after exposure to extract 25, ***Oreopananx glabrous***. Figure 9 shows a low magnification of crystals formed by healthy HepG2 cells. Since only healthy cells can effectively metabolize the MTT molecule and produce the blue formazan crystals, the healthier the cell line the more blue crystals formed. Then, it stands to reason the higher the intensity of the blue color in the ISO/BPS solution the more cells live in that well. Thus, figure 9 shows more MTT metabolized, hence more cells seem to be alive, than figure 8.

Only 12 plants shows cytotoxicity against both the cell lines. Figure 10 shows these extract and their percent killing from both cell types. These extract mainly fall within two plant genuses, Oreopanax and Dendropanax. However, the most cytotoxic of these extracts are shown bellow:

#	Plant Name	HepG2	H4IIE
26	***Dendropanax quercettii***	98.5±0.12	100±1.12
28	***Oreopanax nubigenum***	96.98±0.23	98.6±0.45
31	***Oreopanax capitatus***	94.7±3.2	100±0.69
32	***Didymapanax pittieri***	92.8±1.1	97.0±0.36
33	***Oreopanax liebmannii***	93.7±1.5	98.6±0.18
35	***Orepanax oerstedianum***	93.8±0.79	90.6±1.31
36	***Dendropanax arboreus***	96.6±0.0	97.9±0.45
38	***Dendropanax arboreus*** Neutral fraction	97.2±0.08	97.9±0.064

The ***Dendropanax arboreus*** crude extract was further fractionated into neutral, hexane, alkaloid and phenolic fractions. The neutral fraction seemed to contain the active compound. The individual fractions for ***Dendropanax quercettii***, both the

phenolic and the neutral fraction still contained the active compound. The *Dendropanax gonatopodis* neutral fraction also carried the active compound. Hence it can be assumed that the active compound must have a hydrophilic region where it is attracted to a polar solvent such as water.

An interesting finding was made that the antitumor strength of the each plant species varied based on the season , individual plant and its location. This phenomena may be explained by the hypothesis that the presence of the active compound is based on a trigger mechanism that may or may not be active in all the same plants at the same time. For example, one particular plant may be under fungal attack, and this invasion may have triggered the synthesis of a compound that may not be present in another plant (same specie). Figure 4 shows a comparison between extracts collected from the same plant specie at two different time intervals. The data reveals that the 1992 extracts show an almost uniform decrease in cytotoxicity effect from the 1991 products. A common factor must have caused a decrease in production of the active compound in the 1992 extracts. If this hypothesis is true, perhaps these factors can be manipulated to increase the production of the antitumor compound.

The active compound in *Dendropanax arboreus* has been identified by the University of Alabama in Huntsville Natural Products Research Group. This compound is deadly towards the cancer cells but not towards the normal cells. Therefore, this line of research is promising. There are many other steps ahead of this project yet to be fulfilled and explored. Many plants are yet to be discovered and assayed, and many tests and experiments are to be performed; and although, this

experiment only assayed some of these natural products against two types of cell cultures, it may be a good starting point for a prosperous goal for mankind--a cure for cancer.

Work Cited

1. S.M. Kupchan, S. J. Barboutis, J. R. Knox, and C.A. Lau Cam,*Science* **150** (1965), 1827.

2. Wani, M. C.,Taylor, H. L., Wall, M. E., Coggon, P. & McPhail, A. T. *J. Am. Chem. Soc.* **93** (1971), 2325-2327 .

3. S.M. Kupchan, S. J. Barboutis, J. R. Knox, and C.A. Lau Cam,*Science* **150** (1965), 1827.

4. Cancer census. *Time* 15 Nov 1971:86-87.

Table 1.

#	Plant Extract	Percent Cells Killed	
		HepG2	H4IIE
1	*Schefflera sp.*	14.6±20.72	1.4±5.50
2	*Hieronyma poasana*	6.73±9.53	--
3	*Oreopanax staleyi*	16.7±14.65	23.6±9.22
4	*Persea schiedeura*	7.32±3.75	0.0*
5	*Sapium pachystachy*	2.27±3.95	1.9±20.47
6	*Alchornia latafolia*	41.6±4.58	29.7±1.14
7	*Tetrochidium sp.*	0.0*	3.3±5.6
8	*Oreopanax oerstediuanus*	43.9±12.4	--
9	*Clusia sp. 1990 collorless Sap #1*	60.6±12.6	--
10	*Clusia sp. 1990 Yellow Sap #1*	86.7±1.64	--
11	*Clusia sp. 1990 Collorless Sap #2*	80.6±4.21	--
12	*Clusia sp. Cream Sap #1*	98.8±0.51	--
13	*Clusia sp. 1991 Cream Sap #2*	3.2±6.1	--
14	*Clusia sp. 1991 Megaphyllia*	2.5±1.5	0.0*
15	*Clusia sp. 1991 Colorless Sap #1*	3.2±3.5	0.0*
16	*Clusia sp. Rotuned Leaves*	17.0±2.42	51.2±34.5
17	*Zopote sap Fruit*	7.01±3.17	6.5±2.92
18	*Clusia pettiolate Leaves*	5.9±8.36	0.0*
19	*Ficusa pertusa*	7.8±5.5	0.0*
20	*Ficusa velutina*	1.7±0.82	0.0*
21	*Ficusa tuerkemia*	35.1±7.9	5.1±19.2
22	*Bizzy*	0.0*	44.6±19.3
23	*Malvecea hibiscus*	0.0*	12.7±3.43
24	*Hydrangia sp.*	0.0*	0.4±28.3
25	*Oreopanax globrous*	60.6±14.8	17.5±4.4
26	*Dendropanax guerettii*	98.5±0.12	100±1.12
27	*Oreopanax sanderianus*	62.4±0.45	97.5±0.36
28	*Oreopanax nubigenum*	96.98±0.23	98.6±0.45
29	*Oreopanax sp.*	95.5±0.24	51.4±9.7
30	*Dendropanax gonatopodus*	97.98±0.09	82.9±5.52

*--Numbers may be bellow zero and trunkated.

Table 1. Continued

31	*Oreopanax capitatus*	94.7±3.2	100±0.69
32	*Didymopanax pittieri*	92.8±1.1	97.0±0.36
33	*Oreopanax liemannii*	93.7±1.5	98.6±0.18
34	*Oreopanax xalapensis*	92.7±1.9	20±8.9
35	*Oreopanax oestedianum*	93.8±0.79	90.6±1.31
36	*Dendropanax arboreus*	96.6±0.0*	97.9±0.45
37	*D. arboreus Hexane Fraction*	65.1±3.4	49±15
38	*D. arboreus Neutral Fraction*	97.2±0.08	97.9±.064
39	*D. arboreus Alkaloid Fraction*	--	0.0*
40	*Dandilion sap*	--	95.2±3.25
41	*Dendropanax guercettii*	1.9±13.1	--
42	*D. guercettii Alkaloid Fraction*	--	6.2±8.28
43	*D. guercettii Phenolic Fraction*	--	98.9±0.27
44	*D. guercettii Hexane Fraction*	--	66.3±6.47
45	*D. guercettii Neutral Fraction*	--	98.2±0.36
46	*D. gonatopodis Nuetral Fraction*	--	96.3±2.38
47	*D. gonatipois Alkaloid Fraction*	--	0.0*
48	*Parathesis obovalifolia*	--	11.1±4.83
49	*Amptiitecna haberi*	--	19.4±5.84
50	*Tovomytopsis alienii*	--	7.3±4.56
51	*Tovomytopsis psychotrofotip*	--	97.1±0.46
52	*Near corsetti "largleaf"*	--	83.9±9.73
53	*Clusia "no name"*	--	0.0*
54	*Clusia "clear Sap"*	--	0.0*
55	*Clusia "small fruit"*	--	56.3±20.6
56	*Clusia shiphilla*	--	0.0*
57	*Octea tonduzii*	--	0.0*
58	*Blkea chlorantha*	--	0.0*
59	*Rondetetia monterverdensis*	--	0.0*
60	*Clusia sp.*	--	0.0*
61	*D. arboreus Fraction 1*	--	0.0*
62	*D. arboreus Fraction 2*	--	83.2±7.12
63	*D. arboreus Fraction 4*	--	88.4+3.08
64	*D. arboreus n*	--	0.0*

Figure 1. Cytotoxic effect of extracts on HepG2 cells-Human liver carcinoma.

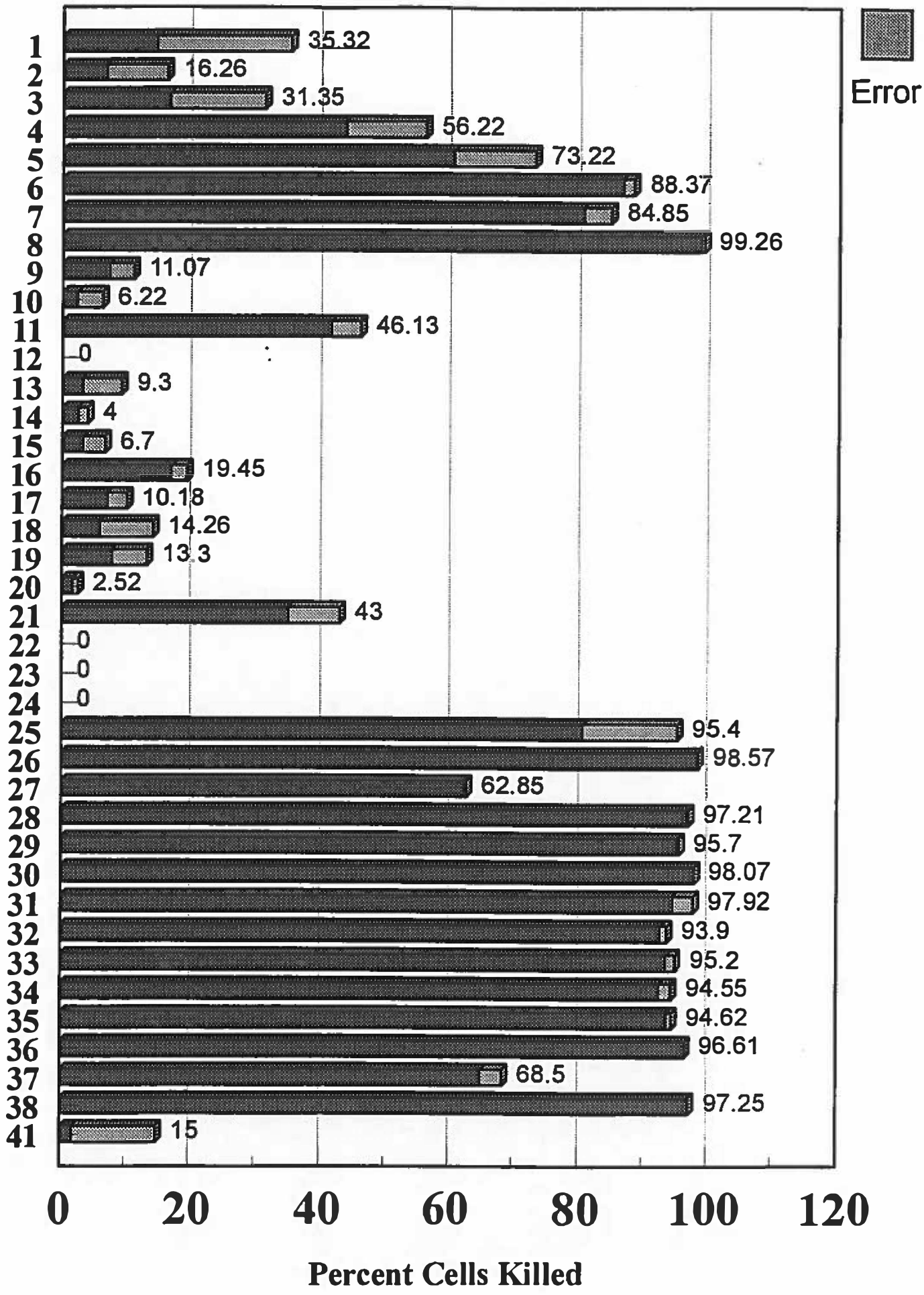


Figure 2. Cytotoxic effect of extracts on H4IIE cells--rat liver carcinoma.

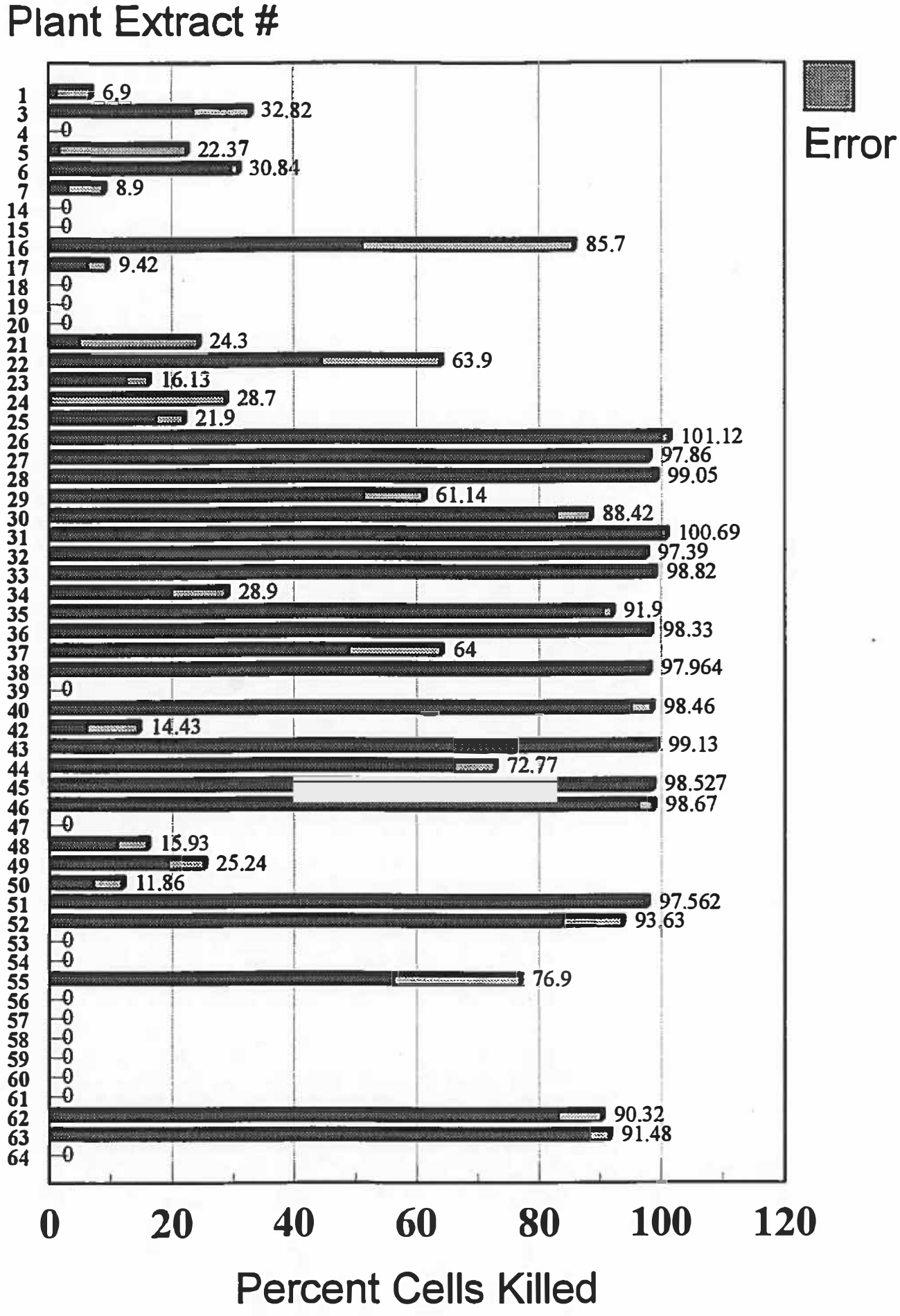


Figure 3. Healthy culture of HepG2 cells.

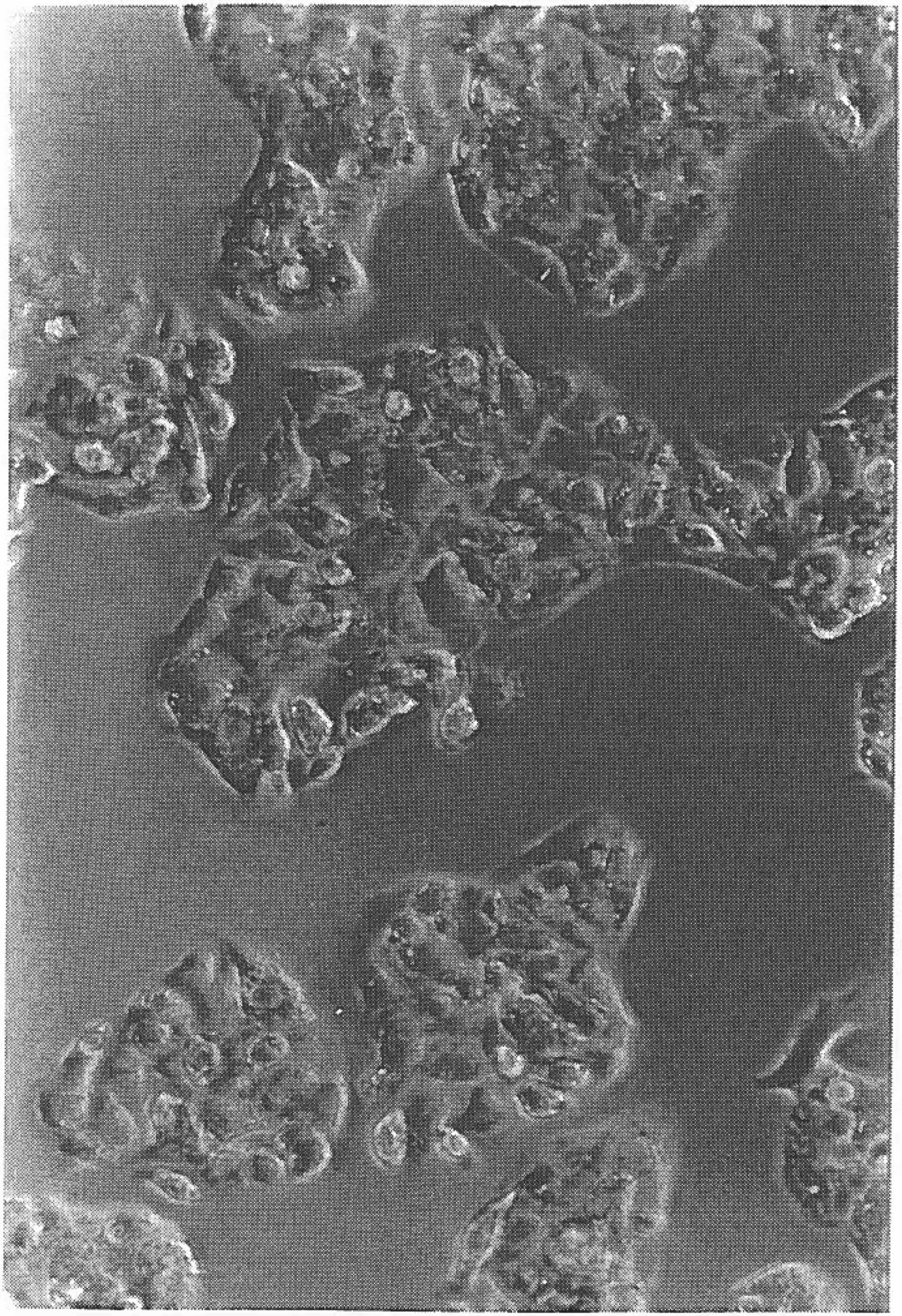


Figure 4. Dead culture of HepG2 cells after exposure to extract 26, *Dendropanax guercettii*.

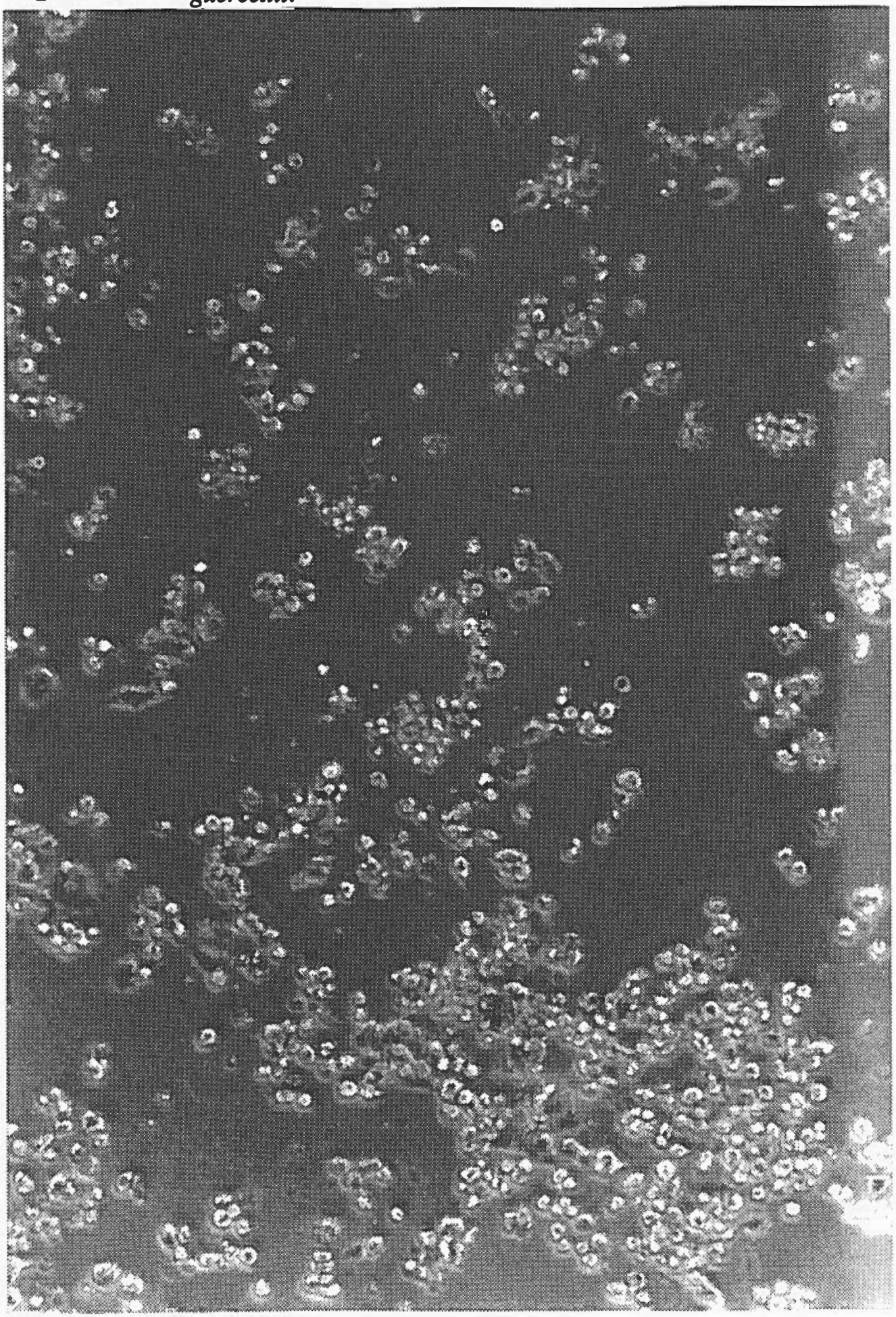


Figure 5. Unhealthy culture of HepG2 cells after exposure to extract 25, *Oreopananx glabrous*.

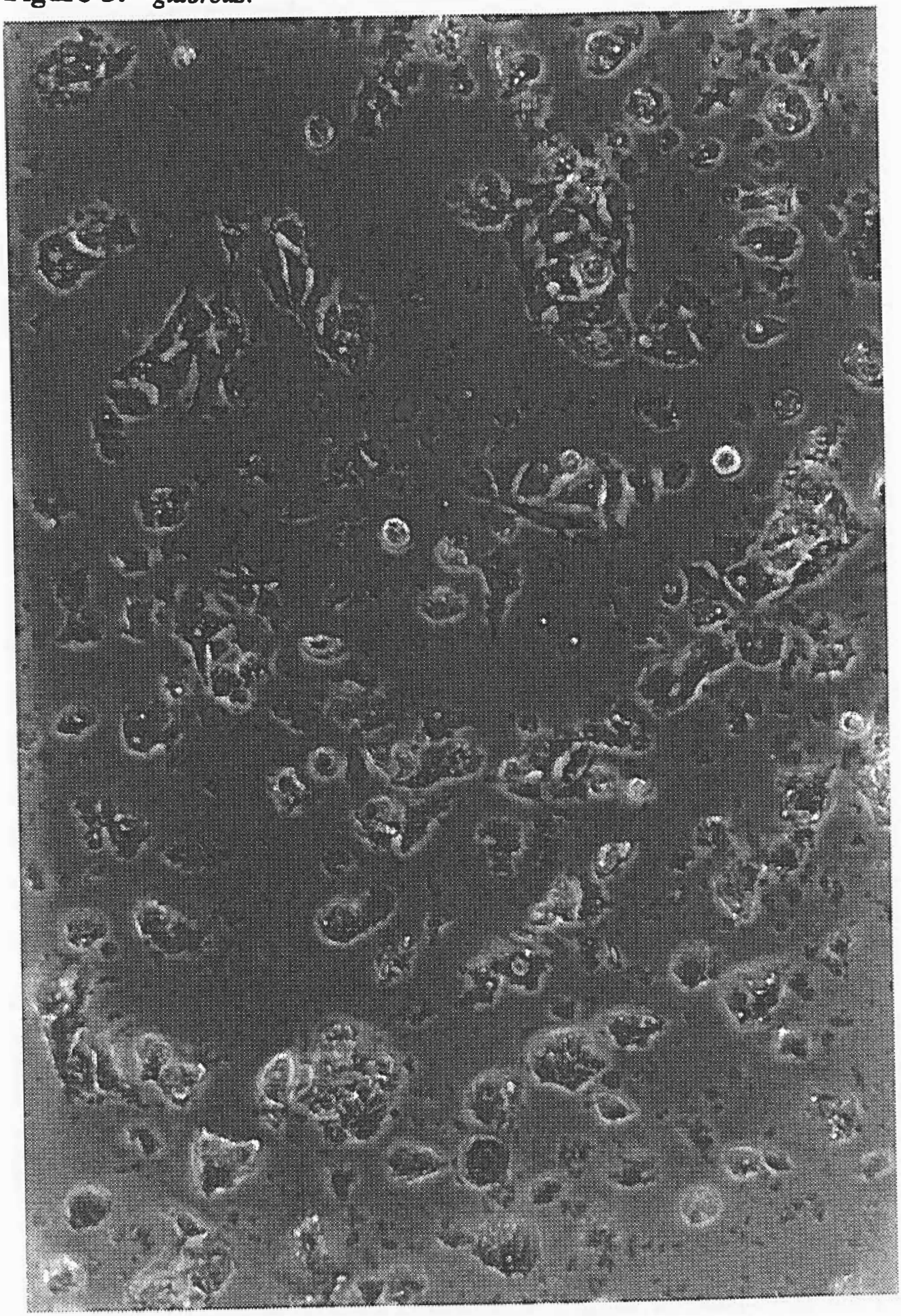


Figure 6. Formazan crystals formed by healthy HepG2 cells after the MTT assay.

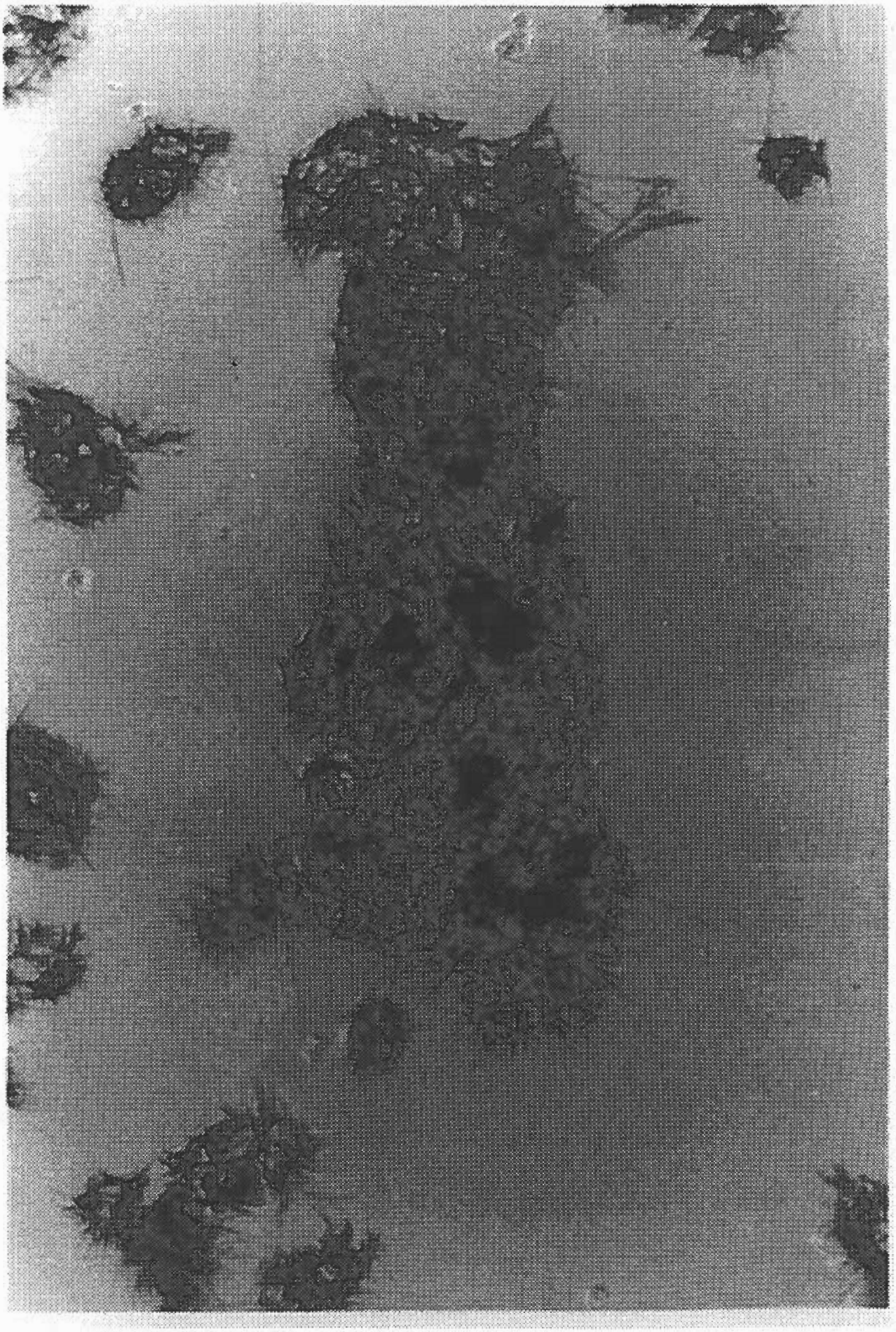


Figure 7. Formazan crystals formed by healthy HepG2 cells after the MTT assay at a higher magnification.

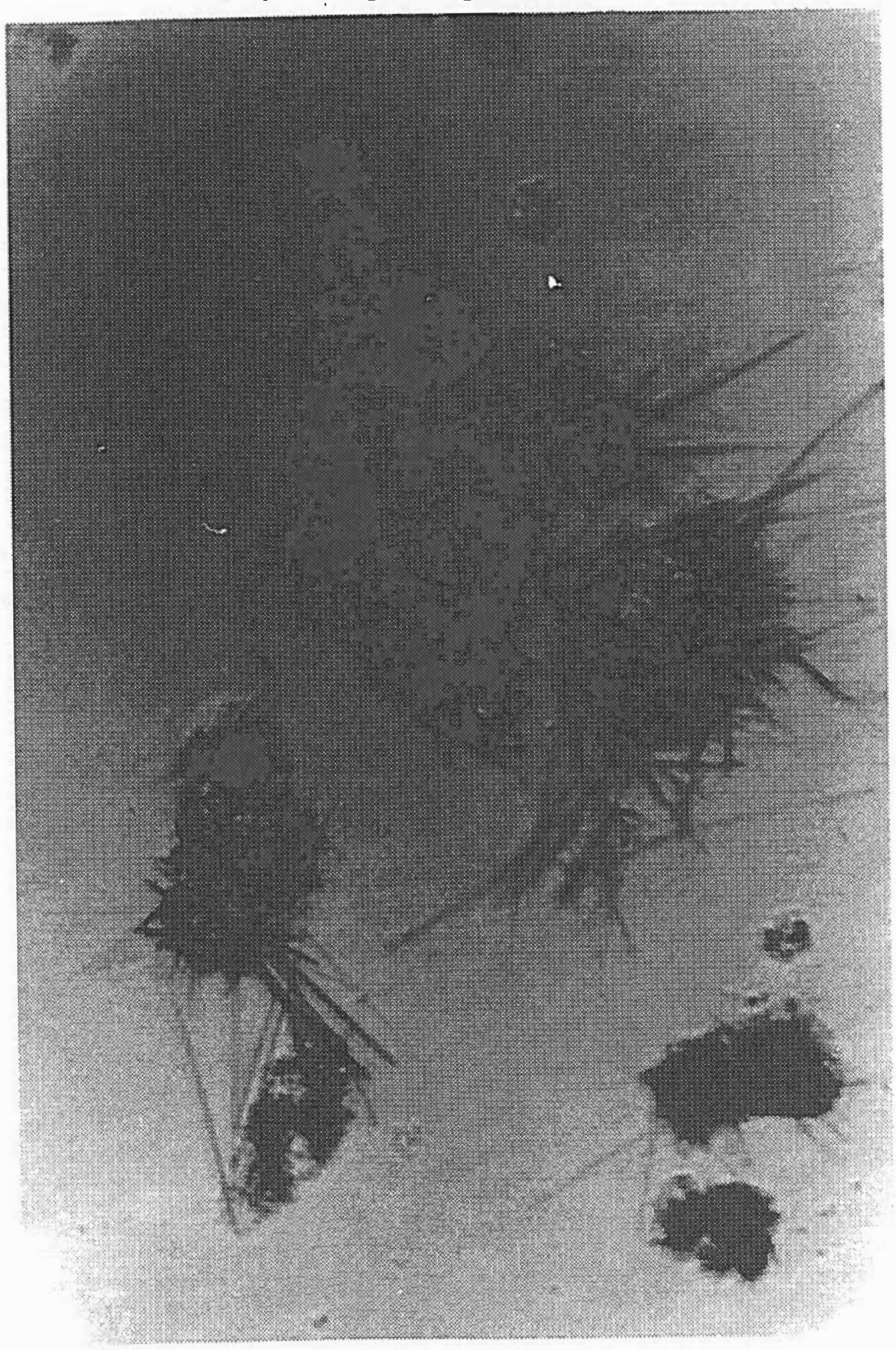


Figure 8. Low magnification of some internal crystals produced by unhealthy HepG2 cells after exposure to extract 25, *Oreopanax glabrous*.

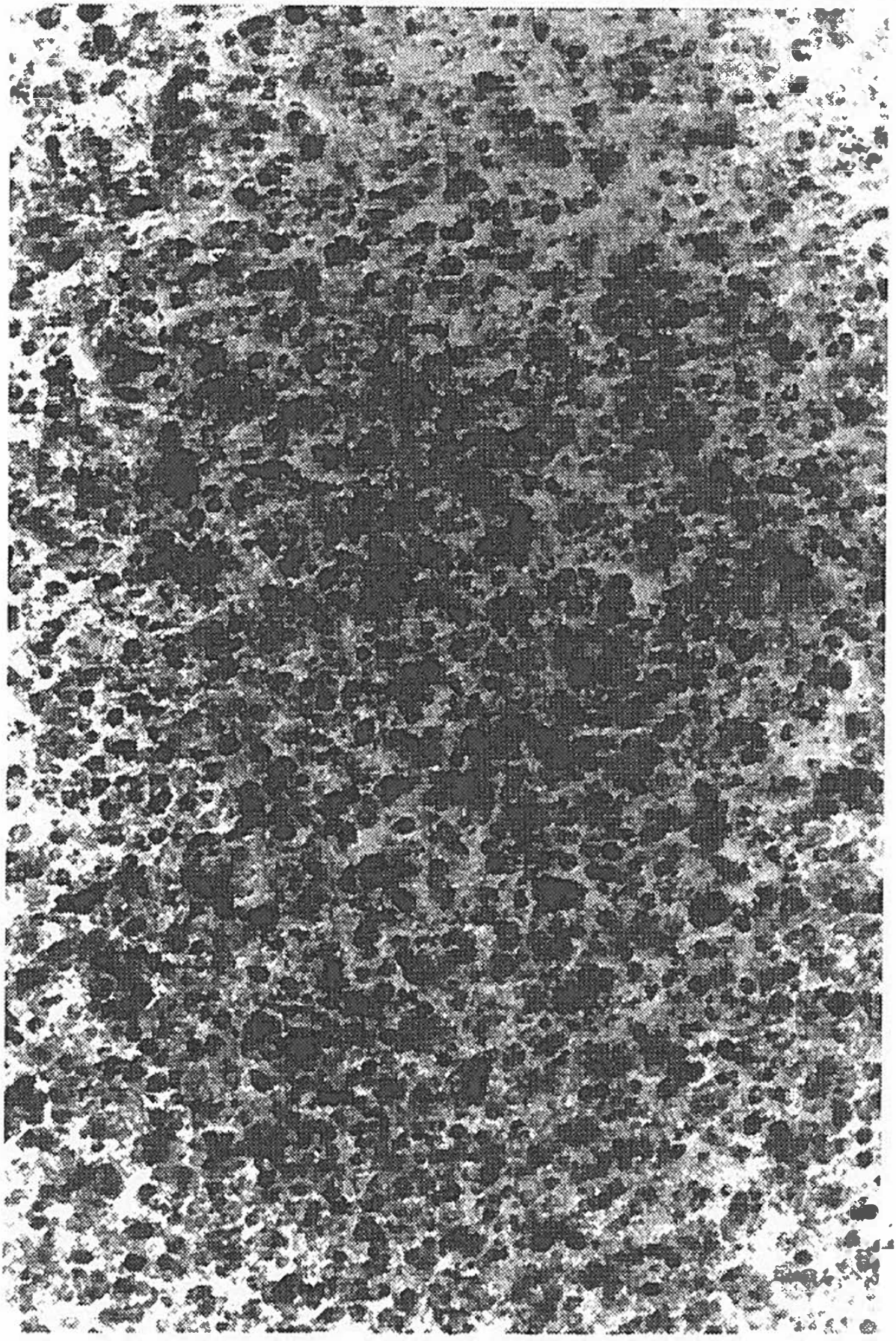


Figure 9. Low magnification of Formazan crystals formed by healthy HepG2 cells

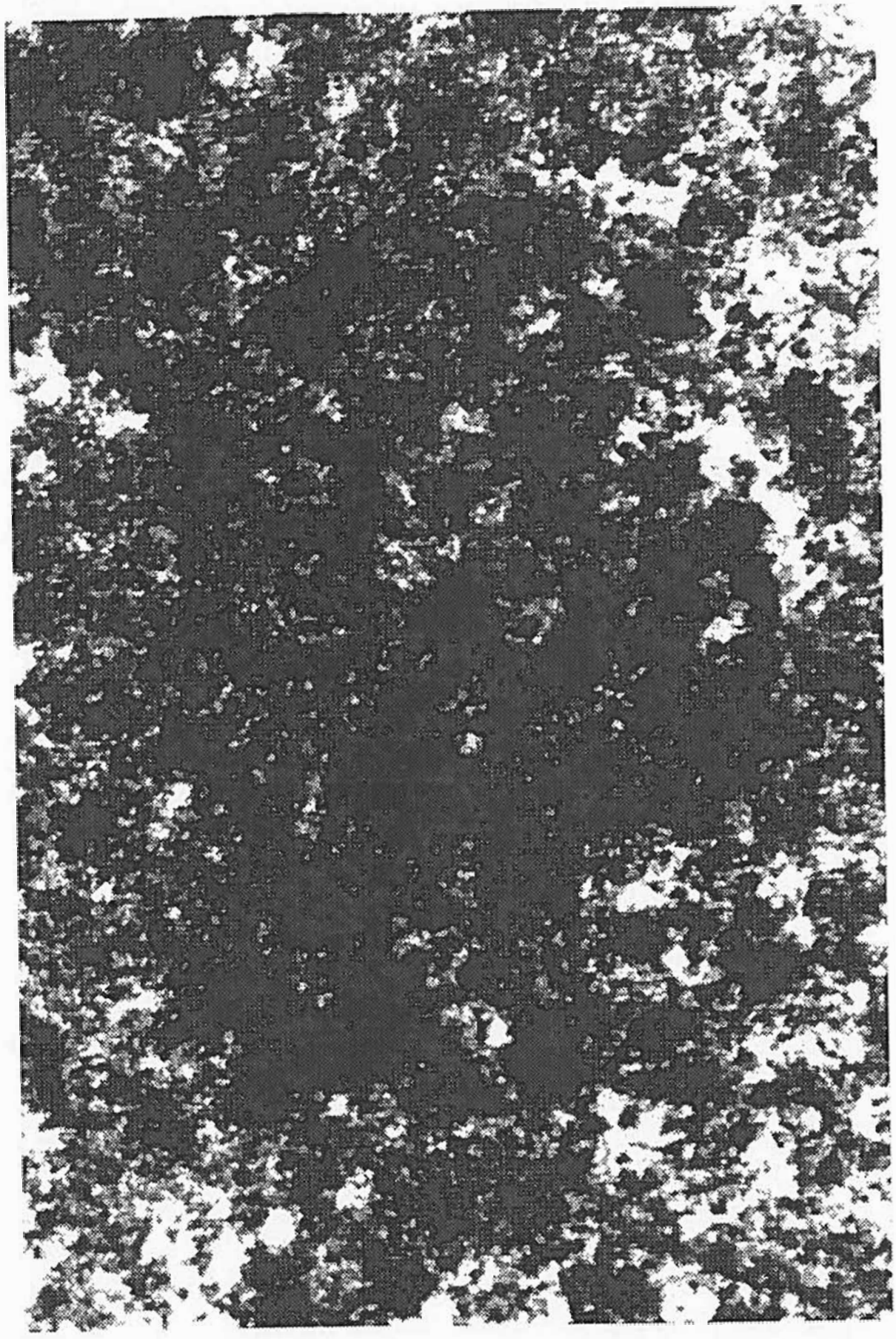


Figure 10. Extracts demonstrating high cytotoxic effect.

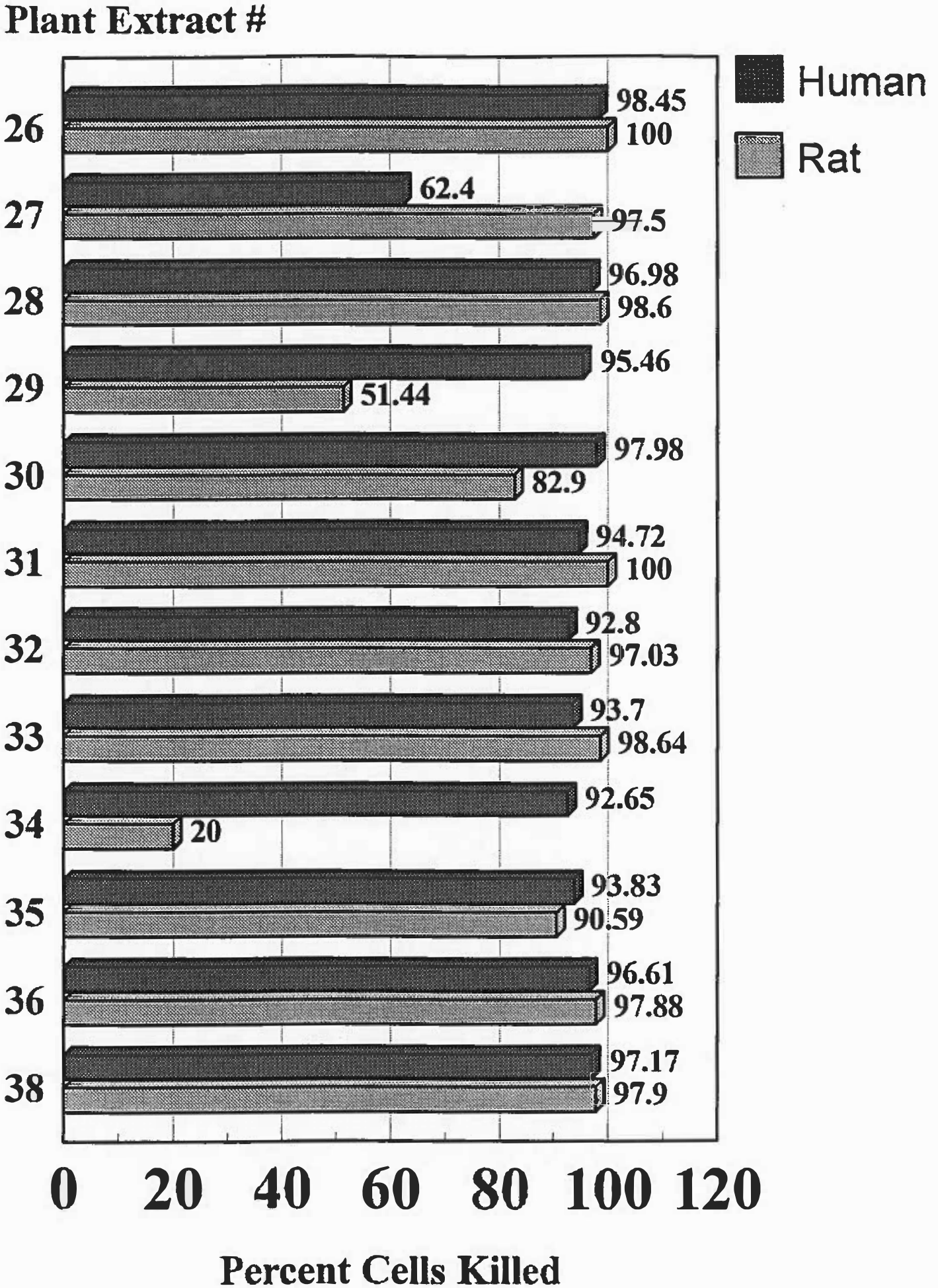
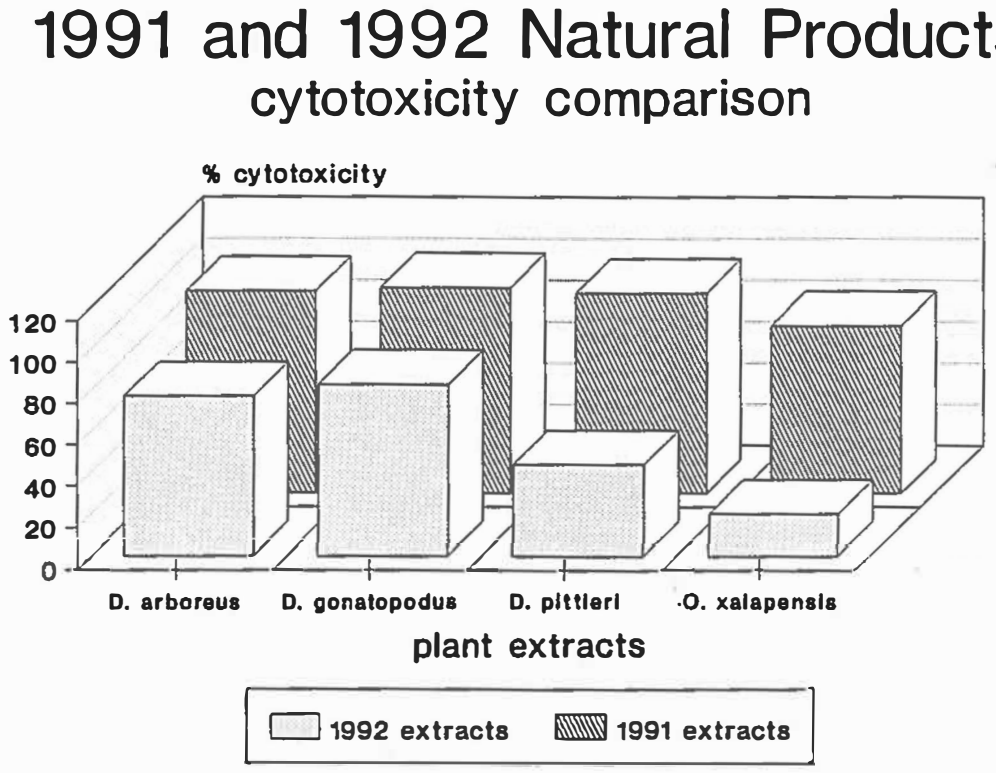


Figure 11. Cytotoxic comparison of extracts from 1991 versus 1992.

Plant extract	% w/v in DMSO	1991 % killed	1992 % killed
Dendropanax arboreus	1.0	97.9	77.1
Dendropanax gonatopodus	1.0	99.7	82.9
Didymopanax pittieri	1.0	97.0	44.0
Oreopanax xalapensis	1.0	81.3	20.0



THE HONORS PROJECT PROPOSAL

PROPOSED TITLE: Antitumor Cell Compounds from Neotropical Plants

STUDENT: Mark Tafazoli

STUDENT #: 077729 **PHONE:** (205)-882-2932

ADDRESS: 1000 Airport Rd. A-27 Huntsville AL 35802

THIS PROJECT FULFILLS REQUIREMENTS FOR THE UAH HONORS PROGRAM &

DEPARTMENTAL & COURSE CREDIT IN BYS 499H

PROPOSAL APPROVED BY:

PROJECT ADVISOR: Debra M. Moriarity **DATE:** 3/1/93

DEPARTMENT CHAIR: R. S. Campbell **DATE:** 3/2/93

DATE PROPOSAL RECEIVED IN HONORS OFFICE _____

DATE PROPOSAL APPROVED BY HONORS COUNCIL _____

SIGNATURE OF HONORS DIRECTOR _____

ATTACHMENT 1

Honors Program
Senior Research Project
Preliminary Description

Name Babador Tafazoli

Student Number 022229

1. **Brief Working Title of your Proposal:**

   Anti-Cancer Drug Research

2. **Attach a two or three paragraph description of the subject of your proposed project:**

   See proposal

3. **In which term/academic year do you plan to complete your project:** Spring 1993

4. **Name(s) and department(s) of possible advisors:**

   Dep. of Biology Dr. Debra Moriarty
   Dep. of Chemistry Dr. Clyde Riley

5. **Which courses or other experiences do you consider necessary preparations for your project:**

   Completed work: Biochemistry, Instrumental analysis, Bio-physical chemistry, Cell Biology, Organic chemistry, Micro-biology + Research/Lab technician for Dr. Riley + Dr. Hausmann.

Planned Courses:

Course	Term to be Taken
# 444	Spring 1993

THE REMAINDER OF THE PROPOSAL CONSISTS OF THREE SECTIONS AND A PRELIMINARY BIBLIOGRAPHY. ADDRESS EACH SECTION IN TURN, USING THESE SECTION HEADINGS IN YOUR DISCUSSION TO CLEARLY INDICATE EACH PART OF THE PROPOSAL. CONFINE YOUR DISCUSSION IN EACH SECTION TO NO MORE THAN ONE SINGLE-SPACED TYPED PAGE. USE THE ATTACHED FORM FOR THE BIBLIOGRAPHY.

PLEASE WORK WITH YOUR PROJECT ADVISER TO ADAPT THE TERMINOLOGY POSED IN EACH SECTION TO YOUR PARTICULAR DISCIPLINE.

I. BACKGROUND/LITERATURE REVIEW. Summarize the "body of know˜edge" or range of perspectives that inform your particular research topic. Be specific in terms of the contributions of individual researchers, theorists, methodologists, critics, etc., to your line of inquiry.

II. RESEARCH QUESTION. How does your work relate to the background you've discussed above? What i˜ the particular question or theme that you will address and how do you expect it to contribute to the inquiry in this field? Do you have a working hypothesis or perspective?

III: METHODOLOGY/APPROACH. How will you go about addressing your question? Be specific in terms of research design, statistical procedures, analysis of primary texts, use of archival sources or data bases, etc., as appropriate to your discipline.

I. Background:

Medical botany is designed to bring into perspective the massive knowledge acquired by man to retain his health by using the plants around him. Man's survival has been dependent upon his innate curiosity, his desire to examine by trial and error all aspects of his environment, and to conclude, for example, which materials are remedial, which ones are harmful, and which give him the greatest nourishment. This legacy exists today. Many of today's pharmacological drugs are based on natural products. For example, willow tree bark contains Aspirin. Ancient doctors asked their patients to chew on fresh willow tree branches in order to alleviate pain. *S. dulcamara,* a tropical plant, has been used to treat cancer, tumors, and warts from the time of Galen (A.D. 180); in addition, references to its use have appeared in the literature of many countries[1]. Taxol is another isolated compound from the plant *Taxus brevifolia* which shows antitumor activity[2]. Such "historical remedies" have led to the discovery of many pharmacological drugs.

Due to advances in science and technology, today many of these drugs can be manufactured synthetically. With sophisticated techniques and devices, the active compounds can be purified and the structures accurately determined. Additional research can inform the scientists about mechanisms or metabolic actions of the drugs. Modern miracle drugs evolve in this manner.

The originally discovered natural product may then pave a way for discovering a new cancer drug. Thus, much research and preparation follows the discovery of such naturally produced drugs.

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[1] S.M. Kupchan, S. J. Barboutis, J. R. Knox, and C.A. Lau Cam, *Science 150* (1965), 1827.

[2] Wani, M. C.,Taylor, H. L., Wall, M. E., Coggon, P. & McPhail, A. T. *J. Am. Chem. Soc.* 93, 2325-2327(1971).

II. Research Question:

The objective of this research was to find and isolate natural organic compounds from tropical plants that have potential antitumor activity. Several criteria were used for the selection of tropical plants. The first being that several successful findings have been cited from plants in these tropical forests in the past[3]. Rain forests have humid climates that are suitable for bacterial and fungal growth, and many plants have to protect themselves against these microorganisms. As one measure of protection plants synthesize organic compounds that are either toxic or retard the growth rate of many of these rapidly growing organisms. Other fast growing cells such as tumor cells may also be retarded or killed upon exposure to some of these compounds. Since theses tropical forests contain plants that may have never been discovered, they make an even better candidate for research.

This project can be a part of the war against cancer, in which it can lead to a cure for it. Once a plant extract is found to inhibit the growth of cancer cells, it can be further studied to find the chemical compound responsible for its effect. Organic synthesis of the compound and further structural modifications may also be performed to possibly optimize its effect. After further animal testing, pharmaceutical companies may in fact one day commercially industrialize a drug.

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[3] S.M. Kupchan, S. J. Barboutis, J. R. Knox, and C.A. Lau Cam, *Science 150* (1965), 1827.

III. Methods and Approach:

The preliminary screening of many of these extracted compounds was an essential part of the research. Compounds were extracted from over 60 tropical plants of Monteverde Cloud Forest Reserve in Costa Rica. A crude extract was obtained using 90% ethyl alcohol, which was later evaporated and extracts were redissolved in Dimethyl sulfoxide (DMSO). A Human liver carcinoma cell line was maintained and the plant extracts were introduced to the cells. The viability of the cancer cells after exposure to the extracts were determined using an MTT assay. MTT assay is a standard technique in which the viability of cells can be detected. Only live cells will consume the MTT molecule and will accumulate a blue byproduct inside. By comparing the intensity of colors of the cell cultures, one can determine to what extent the different extracts inhibited the growth of the cells.

Plant extracts that did show considerable cytotoxicity against cancer cells were fractionated by a High Performance Liquid Chromatography (HPLC). This step separated the compounds in the extract based upon their size and Molecular weights. The fractions were further assayed for cytotoxicity using the MTT assay.

This portion of the research has already been fulfilled. To complete the research the fractions that did show anti-cancer activity will be further fractionated, assayed, and purified. And, the final aim of this research would be to determine the structure of the chemical compound(s) present in the extracts responsible for the cytotoxicity against the cancer cells.