EVALUATION OF PLANT EXTRACTS FOR CYTOKINE MIMETIC PROPERTIES

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Abstract

Extracts of forty-five (45) local plant species were evaluated for their proliferative effects towards mouse spleen cells and bone marrow cells to identify indigenous plants that can be used as source of cytokine mimetics. The activity of the plant extracts was assayed *in vitro* by growing the indicator cells in 96-well tissue culture plates for three days. Results revealed that the extract of the leaves and roots of sweet potato (*Ipomoea batatas*), yam (*Dioscorea alata*), the leaves of buyo (*Piper betle*) and garlic (*Allium sativum*) extracts were very effective in promoting proliferation of both splenocytes and bone marrow cells, increasing the cell concentration 5 to 10 times. Extracts of taro (*Colocasia esculenta*) leaves and roots and cassava (*Manihot esculenta*) leaves stimulated growth of spleen cells only but not the bone marrow cells. The optimum concentration of protein required to exhibit maximum biological response ranged from 1.25 micrograms to 5.0 micrograms per 100 microliter of culture medium and the mimetic activity of the six months stored promising extracts did not significantly reduce suggesting that the activity of the extracts were stable.

Introduction

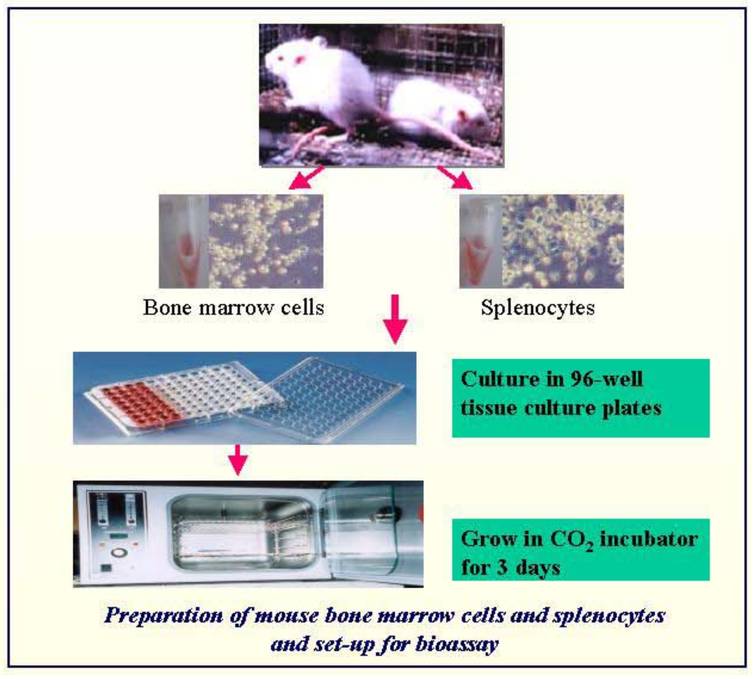
Cytokines represent an array of secreted regulatory proteins that control the survival, growth, differentiation and effector function of tissue cells. Cytokine mimetics are molecules that show similar biological effects of cytokines. Aside from being produced in humans and animals, molecules that display similar and even more potent activity than cytokines can be derived from plants. The Philippines abounds with plants which have been known to possess properties ranging from analgesic to anti-bacterial activities. Some plant species which are popularly known as medicinal plants have also been implicated to have curative effects against major clinical disorders. While it will be very expensive for the Philippines being a developing economy to join the race of biomedical research, nevertheless it is very attractive to explore the potentials of our native plant species as sources of materials with clinical importance/application. This work provides a knowledge database of the properties of our indigenous plants with potential promise for direct clinical use, further exploration and intensive investigation.

Objectives

This study was conducted to identify Philippine indigenous plant species whose extract preparations can be used as source of cytokine mimetics and to evaluate the biological activity and stability of the crude extract preparation from candidate plant sources.

Methodology

Preparation of Cellular Extracts for Protein Expression Analysis

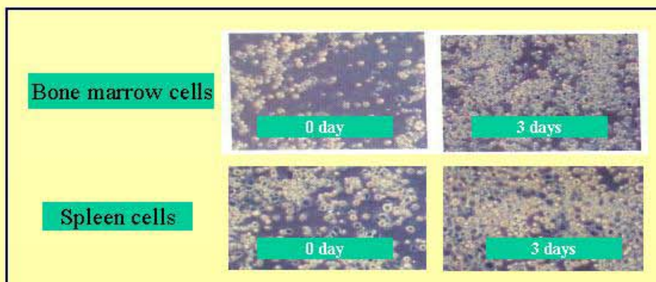


Preparation of mouse bone marrow cells and splenocytes and set-up for bioassay

Results

Table 1. ***Candidate plant sources with potent proliferative activity (Cell count of bone marrow and spleen cells after 3 days incubation. Data are expressed as 10^4. Initial cell number cultured was $1x10^4$)***

Plant Extracts	Conc. of plant extracts (ug total protein)					
	0	0.63	1.25	2.5	5.0	10.0
A. Bone marrow cells						
Sweetpotato leaves	-	-	-	5.62	-	-
Taro roots	-	-	5.62	9.37	-	-
Singkamas leaves	-	-	29.25	25.75	20.25	-
Yam leaves	-	-	39.25	34.75	27.12	-
Noni fruit	-	-	-	35.62	31.00	-
Guava	-	9.50	-	29.75	43.37	-
Garlic	-	50.50	40.75	45.12	32.62	-
Papaya	-	-	40.62	33.75	-	-
Manzanilla	-	-	41.87	37.25	-	-
Buyo		30.12	37.12	27.87	27.37	11.0
B. Splenocytes						
Sweetpotato leaves	-	6.42	9.46	13.26	15.53	-
Sweetpotato roots	-	3.07	-	9.11	-	-
Taro leaves	-	-	1.30	1.50	-	-
Yautia roots	-	-	14.73	5.61	-	-
Iba	-	11.23	9.88	10.38	6.23	-
Onion	-	4.57	2.60	-	-	-
Manzanilla	-	-	-	5.38	8.38	-
Ampalaya leaves	-	-	14.15	4.23	-	-
IL-3	-	16.65	12.76	14.96	14.03	-



Phenotypes of bone marrow cells and splenocytes at 0 and 3 days of culture.

Table 2. ***Under conditions of stress (hypoxic conditions). Cell count of bone marrow and spleen cells after 3 days of incubation. Data are expressed as 10^4. Initial cell number cultured was $1x10^4$.***

Plant Extracts	Conc. of plant extracts (ug total protein)					
	0	0.63	1.25	2.5	5.0	10.0
Bone marrow cells						
Sweetpotato leaves	-	8.75	11.50	22.50	8.50	-
Sweetpotato roots	-	16.62	18.50	-	24.75	10.12
Yam leaves	-	70.50	51.25	36.87	16.12	59.37
Yam roots	-	71.87	62.00	83.12	65.75	62.37
Splenocytes						
Sweetpotato leaves	-	6.76	15.88	14.15	13.23	15.92
Sweetpotato roots	-	9.92	11.19	10.46	15.69	15.26
Yam roots	-	11.84	13.50	19.73	16.92	1.53
Banaba leaves	-	27.11	10.80	-	-	-
Buyo	-	-	-	10.69	-	-

Significant Findings

1. The leaves and roots of sweet potato (*Ipomoea batatas* L.) and yam (*Dioscorea alata*) were very effective in promoting proliferation of both splenocytes and bone marrow cells *in vitro*, increasing the cell concentration 5 to 10 times the initial number of cells cultured for a duration of three days.
2. Taro (*Colocasia esculenta*) leaves and roots and cassava (*Manihot esculenta*) leaves stimulated growth of spleen cells only but not the bone marrow cells.
3. Interestingly, extracts derived from buyo (*Piper betle*), an indigenous plant that is "chewed by older people in the rural communities and indigenous people in remote areas", was very potent in stimulating growth of both bone marrow cells and splenocytes increasing the cell concentration to nearly 40 times than the initial cell concentration cultured.
4. Extract of garlic (*Allium sativum*), an abundant spice, was also very potent in increasing population of bone marrow cells *in vitro* in a concentration-dependent manner with maximum response observed at 0.63 micrograms per 100 microliter of culture volume.
5. The optimum concentration of protein required to exhibit maximum biological response varied depending on the kind of extract used. In general, extracts with protein concentrations ranging from 1.25 micrograms to 5.0 micrograms per 100 microliter of culture volume was effective.
6. The biological activities of the candidate plant extracts described above were even superior than the response elicited by interleukin-3 (IL-3), the positive control.
7. The mimetic activity of the promising extracts did not significantly reduce even if the extracts were stored frozen for six months suggesting that the extracts activity was stable.

Conclusion

Based on the above results, it can be concluded that sweetpotato, yam, taro, cassava, buyo, and garlic are promising plants that could be used as sources of cytokine mimetics because of their capacity to promote cell proliferation particularly cells of the immune system.

The Six Major Candidate Plants as Sources of Cytokine Mimetics

Recommendation

It is recommended that follow-up studies on the following areas be pursued to validate the results and further support our claims and findings.

- Mouse *in vivo* studies to determine if the results *in vitro* can be confirmed *in vivo*. This is important before possible clinical tests can be done.
- Characterization of candidate plant mimetics in terms of pH and heat stability.

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