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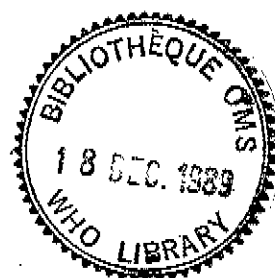
GLOBAL  
PROGRAMME  
ON **AIDS**

AND  
TRADITIONAL  
MEDICINE  
PROGRAMME

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REPORT OF A WHO INFORMAL CONSULTATION  
ON TRADITIONAL MEDICINE AND AIDS:  
*IN VITRO* SCREENING FOR ANTI-HIV ACTIVITY

GENEVA  
6-8 FEBRUARY 1989



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CONTENTS

|   | <u>Page</u> |
|---|-------------|
| SUMMARY .....   | i           |
| 1. Introduction .....   | 1           |
| 2. Ongoing activities in the area of natural products and AIDS.....   | 1           |
| 3. Selection of plants for antiviral screening .....  | 3           |
| 3.1 Selection based on ethnomedical uses .....  | 4           |
| 3.2 Random collection followed by mass screening .....  | 5           |
| 3.3 Follow-up of existing literature leads .....  | 6           |
| 3.4 Chemotaxonomic approach .....   | 6           |
| 4. Procedures for <u>in vitro</u> screening of traditional remedies,<br>medicinal plants and other natural products ..... | 6           |
| 4.1 Authentication of extracts provided for analysis .....  | 7           |
| 4.2 <u>In vitro</u> assays for antiviral activity .....   | 7           |
| 4.3 Assay of "immunoenhancing" activity .....   | 8           |
| 4.4 Further evaluation of promising products .....  | 8           |
| 5. Legal and ethical considerations .....   | 9           |
| 6. General conclusions and recommendations .....  | 9           |
| References .....  | 11          |
| Annex List of participants.....   | 13          |

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## SUMMARY

An informal consultation to rationalize the study of traditional medical products in connection with the problem of AIDS was organized by the Biomedical Research Unit (BMR) of the Global Programme on AIDS (GPA) and the Traditional Medicine Programme (TRM) of the World Health Organization (WHO) and convened at WHO headquarters, Geneva from 6 to 8 February 1989. The consultation was attended by eight participants from five countries (see Annex).

The main elements discussed were: (a) ongoing research and related activities in the area of natural products; (b) procedures for selecting natural products for antiretroviral screening; (c) procedures for *in vitro* screening of natural products; and (d) legal and ethical issues surrounding research in traditional medicine.

Many plant products are being used by AIDS patients and traditional practitioners in Member States without any scientific proof that they possess anti-HIV activity. It was reported that traditional healers are now offering their remedies for scientific evaluation, and this should foster good collaboration in the quest for a treatment for AIDS from natural products.

A few studies have provided information on the inhibitory activity against HIV of plants such as *Viola yedoensis*, *Arctium lappa*, *Epimedium grandiflorum*, *Glycyrrhiza uralensis* and *Castanospermum australe*.

The consultation was in agreement that new initiatives would be warranted in the search for anti-HIV compounds based on their use in traditional medicine. Further, the consultation made recommendations on procedures for selection of natural products with possible anti-HIV activity, and proposed *in vitro* methodologies for screening the extracts.

Natural products can be selected for biological screening based on ethnomedical use, random collection, pursuit of literature leads or a chemotaxonomic approach that entails screening species of the same botanical family for similar compounds. The consultation concluded that follow-up of ethnomedical information and selection of plants based on literature leads, would seem to be the most cost-effective way of identifying plants with anti-HIV activity.

As regards *in vitro* screening methodologies for anti-HIV activity, the consultation noted that a wide range of biochemical and cell culture-based assays are currently available. It was recognized that no one assay is ideal and that confirmatory assays in multiple systems are needed to examine completely the potential use of a compound. However, it is clear that in any assay system, the antiviral effect must outweigh the potential toxicity of the extract or compound.

Noting a lack of clear understanding of the immunoenhancing effect claimed for a large number of traditional medicines, the consultation recommended that immunomodulating extracts be given a lower priority for further evaluation, if the goal is to identify anti-HIV agents.

Having examined the question of legal and ethical issues, the consultation advised that investigators should take appropriate steps to ensure compensation and credit for communities that supply information on traditional medicines in case of discovery of an anti-HIV drug.

To promote further research in traditional medicine and AIDS, WHO will identify appropriate institutions where the different activities needed for the scientific evaluation of traditional medicine relative to AIDS treatment, such as collection of plants, extraction, *in vitro* and *in vivo* evaluation, and elucidation of structure of active principles can be carried out.

## 1. Introduction

Traditional medicines are being used empirically in many countries in the treatment of AIDS. Evaluating these treatments in HIV-infected individuals is a new challenge which confronts the world today. With the present global estimate of 5-10 million people currently infected with HIV, the need for effective treatments is pre-eminent. All possible resources must be made available for the benefit of affected populations. In this context, there is a need to evaluate those elements of traditional medicine, especially medicinal plants and other natural products, that might yield effective and affordable therapeutic agents. This will require a systematic approach.

Against this background, the Biomedical Research Unit (BMR) of the Global Programme on AIDS (GPA) and the Traditional Medicine Programme (TRM) of the World Health Organization (WHO) convened an informal consultation in Geneva, from 6 to 8 February 1989, with the following objectives:

- (1) to review available information on the status of research in traditional medicine as applied to HIV infection and AIDS;
- (2) to carry out an independent scientific review of current activities in the area of medicinal plants and other natural products related to in vitro and in vivo preclinical evaluation for antiretroviral or anti-reverse-transcriptase activities;
- (3) to identify opportunities for collaborative work;
- (4) to make recommendations on the main directions of research relevant to the goals of the Global Programme on AIDS.

The informal consultation was attended by eight participants from five countries (see Annex). The meeting was opened by Dr Jonathan Mann, Director of the WHO Global Programme on AIDS. He reminded the participants of the daunting global AIDS situation, and the need to rapidly find an effective, affordable treatment. He emphasized that discussions during the consultation should not be limited to medicinal plants, but should also cover other natural products.

Dr Olayiwola Akerele, Programme Manager of the WHO Traditional Medicine Programme, also welcomed participants and expressed pleasure at the opportunity to work with the group in preparing a plan of action for the selection of medicinal plants for initial anti-HIV screening. He also outlined the objective and activities of the WHO Traditional Medicine Programme. Dr José Esparza, Acting Chief of the GPA Biomedical Research Unit (BMR), briefly presented the research philosophy and strategies of BMR, in support of control measures for AIDS. Dr Harry Osore explained the method of work. Dr Norman Farnsworth was elected as Chairman, Dr Lu Weibo and Dr Jerome Msonthi as Co-Rapporteurs.

## 2. Ongoing activities in the area of natural products and AIDS

A great many plant products are presently being used by AIDS patients without any experimental evidence of anti-HIV activity. These include the following: garlic (Allium sativum), shiitake mushrooms (Lentinus edodes), papaya (Carica papaya), ginseng (Panax species), Aloe vera, ukrain (Chelidonium majus), immunact (a Peruvian plant root), Japanese pine cone extract, various flower essences, Easter lily bulbs, Fu-zheng (a Chinese traditional principle of treatment), and Padma 28 (a Tibetan formula of several plants). A number of other marine, fungal and animal products have also been used.

Traditional medicines are being employed for the treatment of AIDS in all WHO regions. In many African countries, for example, patients often go to hospital where a clinical diagnosis of AIDS is made; when they see no change in their condition the majority voluntarily leave hospital to seek alternative treatment. Such patients often then go to traditional healers. In some cases, follow-up studies are carried out by physicians in AIDS clinics and counselling centres. Generally, traditional healers voluntarily come forward to offer their remedies for scientific evaluation, especially when they see that patients on such therapies continue to be maintained in a reasonable state.

Several plants are used in AIDS treatment in Africa, and observations have shown that certain plants appear to be common to most of the infusions or concoctions given to patients. Some of these have shown biological activities which qualify them as candidates for further study for anti-HIV activity. For example, Diospyros usambarensis (Ebenaceae) is one such plant that has been found to possess fungicidal and cytotoxic properties.

It was reported to the consultation that Traditional Chinese Medicine is being used in patients with AIDS in Tanzania, with the collaboration of Chinese and Tanzanian scientists. According to this information, the research protocol stipulates that every year, for three years, 200 AIDS patients at different stages of the disease would be treated, with the same number of patients to be used as controls. The patients would be diagnosed and followed-up clinically and for several immunological and serological parameters. The treatment would last for three months, and patients would be treated in accordance with the syndrome differentiation and typing used in Traditional Chinese Medicine, which classifies AIDS patients into four types. A basic recipe is used for each type. During the trial, these recipes could be modified depending on the clinical manifestations. The basic recipe is modified by the addition of one or more of the following: Polyporus umbellatus, Cordyceps sinensis and Paeonia obovata. A special outpatient clinic has been set up for follow-up studies of discharged patients. At present, immunological parameters to determine T-lymphocyte subsets have been established and are being monitored. So far, 17 patients have been treated and some symptomatic improvement claimed.

Only a few experimental studies to discover anti-HIV agents from medicinal plants and other natural products are in progress. The major programme of this type is being carried out at the National Cancer Institute in the United States of America. The programme will screen about 4500 plant samples per year for the next five years for in vitro anti-HIV activity, based on a random selection of plants. No traditional medicine background information has been used in this programme.

It has been reported recently that 11 of 27 medicinal herbs used in Traditional Chinese Medicine as anti-infectives, showed in vitro inhibitory activity against HIV. These were: Arctium lappa, Epimedium grandiflorum, Lonicera japonica, Woodwardia unigemmata, Viola yedoensis, Senecio scandens, Andrographis paniculata, Coptis chinensis, Prunella vulgaris, Lithospermum erythrorhizon and Alternanthera philoxeroides. The active principles of Viola yedoensis and Prunella vulgaris appear to be sulfonated polysaccharides (1, 2).

Other studies have provided additional information on natural products with experimental anti-HIV activity, including glycyrrhizin (from Glycyrrhiza uralensis (3)). In Japan, glycyrrhizin has been studied in AIDS patients. It was claimed that, when given orally to asymptomatic HIV carriers, glycyrrhizin delayed the progression of symptoms related to HIV infection. It was also claimed that relatively large doses of glycyrrhizin administered to AIDS patients caused a disappearance of HIV antigenemia and an improvement in several haematological and immunological parameters. In another claim, simultaneous administration of glycyrrhizin appeared to decrease the adverse effects of Zidovudine.

An aqueous extract of the marine red alga Schizomenia pacifica, was shown to inhibit reverse transcriptase and the activity was attributed to a sulfated polysaccharide. Apparently, many sulfated polysaccharides inhibit HIV activity (e.g., heparin, dextran sulfate) but the non-sulfated polysaccharides (e.g., chondroitin, alginic acid, keratin, and hyaluronic acid) do not (4).

Castanospermine, an indolizidine alkaloid extracted from the seeds of Castanospermum australe, blocks glycoprotein processing via inhibition of glucosidase I located in the endoplasmic reticulum. This alkaloid has been reported to have in vitro anti-HIV activity and has been shown to be active in vivo when administered orally to mice (5).

From the discussions that followed the presentations, it was agreed that new initiatives would be fruitful in the search for anti-HIV compounds in plants, based on their uses in traditional medicine and other natural products. Further, it was recognized that there is a need for collection and analysis of information based on ethnomedical and experimental reports, which include in vitro and in vivo bioassay test models, and on chemical data.

A deficiency of the ethnomedical approach is the lack of specific information on plants that may have anti-HIV activity. The following symptoms might be mentioned in order to secure relevant information from traditional healers: skin lesions (Kaposi's sarcoma), chronic fever, diarrhoea, cough, haemoptysis, and genital ulcers.

### 3. Selection of plants for antiviral screening

Any approach to be used for identifying specific plants to be evaluated is dependent on a number of factors, e.g., simplicity, speed, cost, reproducibility, lack of interference by ubiquitous substances found in plants, and availability of plant material (including the possibility of providing scaled-up quantities of active plants for isolation studies).

Generally speaking, only a small amount of plant material is required initially for most in vitro screens, i.e., 100-200 g dry weight (or less) of the appropriate plant part. Proper documentation of the samples selected by qualified botanists must be made, including collection of voucher specimens. Samples must be dried properly. Care should be taken to avoid collection of threatened or endangered species. Normally, two types of extracts for each sample should be made -- one with a non-polar (or intermediate polar) solvent and one with a more polar solvent, e.g., ethyl acetate or chloroform, and methanol or ethanol, respectively.

Four basic methods are available for selecting plants for a screening programme to seek anti-HIV leads: (a) follow-up of ethnomedical information, (b) random collection of plants followed by bioassay, (c) selection of plants already reported in the literature to have properties that would suggest inhibitory activity against HIV, and (d) chemotaxonomic approaches. All factors considered, approaches (a) and (c) would seem to be the most cost-effective for identifying plants with anti-HIV activity. The advantages and disadvantages of the various approaches to the selection of plants for screening to discover anticancer activity can be compared, as indicated in Table 1. The same advantages and disadvantages would be associated with approaches to discover anti-HIV activity in plants.

Problems associated with a screening programme of this type are: (i) geopolitical; (ii) selection of an appropriate assay or assays that will respond specifically to anti-HIV activity rather than unspecifically to ubiquitous plant constituents; (iii) more than one extract should be made of each plant sample, with costs increasing as each additional type of extract is tested; and (iv) the solvent used in preparing the extracts has to be compatible with the assay system being used.

### 3.1 Selection based on ethnomedical uses

Several terms are used to indicate the use of plants by indigenous peoples, including ethnobotany, ethnomedicine, folklore, and traditional medicine.

Information derived by qualified observers in the field, in which the observer actually sees the plant being used, is the most convincing. Some observers simply question a traditional practitioner for information and record the claims for various plants. Most observers to date do not appear to have had a background in pharmacology or medicine, which raises questions about the validity of many ethnomedical claims found in the literature. For example, a claim that a plant is useful as a "contraceptive" could mean that the plant is used to "prevent conception" (rarely is it stated if use is by men or women) or to "prevent birth" (as an abortifacient, for example). Setting up a bioassay to demonstrate prevention of conception would of course be different from setting one up to show an abortifacient effect. Information derived from an observer who actually sees the use of the plant for a specific disease is obviously more valid than information simply collected as being claimed to have a use by an informant. Similarly, information from a recognized medical system such as Traditional Chinese Medicine, which is based on a written history of 3000 years, and on a system that has a theory and an educational system, is probably more valid than information from an anecdotal book written by one having no background in pharmacology or medicine.

In spite of these apparent problems and the lack of uniformity of information available on the ethnomedical uses of plants, a careful analysis of information of this type should lead to an indication of the plants that would be the most likely to show a positive response in a bioassay system designed to pick up an effect predictive of the clinical situation. Of 121 drugs currently used globally which are obtained from higher plants, 74% were discovered by scientists investigating the plants on the basis of ethnomedical claims (6).

The consultation considered problems that might be encountered in the questioning of traditional healers in an attempt to identify promising leads. In such questioning it will be necessary to make use of clinical signs and symptoms induced by viral pathogens other than HIV. One possibility that was discussed concerns hepatitis B virus, which recently has been shown to contain reverse transcriptase. Additionally, however, hepatitis B virus induces an easily recognizable clinical sign, namely jaundice. In contrast, other viral illnesses produce a variety of clinical syndromes with relatively nonspecific clinical manifestations, which may make it very difficult to obtain information from traditional medical healers about the use of specific agents for treatment of viral illnesses.

In summary, searching for information on potential antiviral effects among indigenous people, i.e., from traditional medicine, will have to take into consideration not only the relatively recent onset of HIV infection, but also the many different clinical signs and symptoms observed in AIDS patients. These considerations could limit the effectiveness of this approach. Such correlations would have to be further studied and defined.

The obtaining of information from indigenous practitioners through the network of WHO Collaborating Centres for Traditional Medicine would seem to be a logical approach. In the case of identifying plants useful as antiviral agents, the ethnomedical data for virus maladies may not bear a strong relationship to in vitro test results. However, it may be of value to use some of the existing ethnomedical data, in conjunction with other types of published experimental data, to strengthen an inclination to collect and test a given species.

### 3.2 Random collection followed by mass screening

This approach is based on the belief that active compounds for any given disease will eventually be found if sufficient samples are randomly selected and tested. The United States National Cancer Institute used this approach from 1956 to 1981, during which time 32 000 species of flowering plants were collected from various parts of the world. Crude extracts were prepared and these extracts were evaluated in at least one in vitro cytotoxicity test system, and one to several in vivo antitumour systems. About 2%-8% of species tested showed reproducible antitumour activity. Plants were collected under the auspices of botanists of the United States Department of Agriculture (USDA) under contract from the National Cancer Institute. The budget for this was approximately US\$ 450 000 per year for collections and recollections. Experienced research groups in the USA were then contracted to follow up active leads, isolating active principles and elucidating that structure. All of the in vivo testing and most of the in vitro testing was carried out at independent contract laboratories. Agents which showed good activity were considered for further broader in vivo testing, pre-clinical toxicology and subsequently, clinical evaluation.

During the 25-year life of this programme, hundreds of active cytotoxic and/or in vivo antitumour agents were discovered in plants. About 12 reached phase I clinical testing, but none of the plant-derived compounds became generally useful.

The clinically useful plant-derived antitumour agents, vinblastine and vincristine, were developed independently by a pharmaceutical company, and were discovered as a result of plants being subjected to a broad screening programme. The podophyllotoxin-derived semi-synthetic plant compounds etoposide and teniposide were developed by a European drug company after much basic information on the antitumour properties of podophyllotoxin and its analogues was provided by the National Cancer Institute, following up claims that American Indians used the source plant, Podophyllum peltatum, for skin cancer.

The National Cancer Institute contract programme was disbanded in 1981 and was dormant for several years. However, in 1987 it was rejuvenated on the basis of new testing procedures (selective in vitro cytotoxicity in a broad selection of human tumour cell lines, in lieu of in vivo testing). Contracts have been given under this new programme for the acquisition of 1500 plant samples per year for five years from South-East Asia, 1500 from South America and 1500 from southern Africa. Instructions to botanists collecting plants are that ethnomedical uses relating to cancer should be a priority in making collections, followed by random collection of plants that represent unusual or rare taxa.

At the Central Drug Research Institute in Lucknow, India, there has been an ongoing programme to screen Indian plants for a broad array of biological activities, including antitumour and antiviral effects. A few active plants have been uncovered in this programme, but useful agents have not been identified. The basis for plant collection was primarily random.

A group at the University of Illinois, USA, has published on the screening of

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