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Distr.
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UNIDO/PC/R.15
28 February 1986

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION
UNITED NATIONS DEVELOPMENT PROGRAMME

ENGLISH

PREPARATORY MISSION ON THE VIABILITY OF PRODUCING PLANT-DERIVED PHARMACEUTICALS AND THE ESTABLISHMENT OF A LABORATORY TO INVESTIGATE THE CHEMISTRY AND THE PHARMACOLOGY OF HERBAL MEDICINE

RP/BOT/85/001

[BOTSWANA . (Plant-derived pharmaceuticals) .]

Terminal report*

Prepared for the Government of Botswana
by the United Nations Industrial Development Organization

Based on the work of Finn Sandberg
UNIDO consultant on plant-derived pharmaceuticals

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Annexure

1. Introduction

This project has the following development objectives:

Production of pharmaceuticals and cosmetics from wild and cultivated medicinal and aromatic plants leading towards improved health conditions and generating rural income.

The expert is - according to the job description - expected to study the practicability and to advise on the manner of implementation of a fully-fledged project for:

(a) the production of plant-derived pharmaceuticals and cosmetics, including the choice of technology for this purpose and the steps needed towards sequential planning of the activities of such a project; and

(b) the establishment of a suitably equipped laboratory to perform chemical and pharmacological assessments on plant material and for quality assessment of products and raw material.

The potential for utilization of medicinal and aromatic plants in Botswana has been evaluated by two previous UNIDO missions: the first one in early 1980, and the second one in 1982 and 1983 by E. Paun. The present expert has read his report, dated 17 June 1983 (UNIDO/IO/R.72). In spite of the serious and solid work, the objectives of these missions were not fully realized for the following reasons:

(a) a suitable infrastructure did not exist at that time; and

(b) the research potentials at the University of Botswana seemed to have escaped attention.

2. Findings

A. General

The schedule for the expert and the persons with whom he has had discussions is found in Annex 1.

The present expert has had several contacts with the University of Botswana, specifically with Dr. John Woollard from the Department of Chemistry.

The fundamental positive change in the infrastructure - needed for this project - is the establishment of THUSANO LEFATSHENG, a non-profit organization, officially registered in January 1984 in the Republic of Botswana with a dynamic General Manager (Mrs. Francoise Horenburg).

The fact has to be recorded that there are at least two key persons with dedication and interest for the proposed project.

The expert has investigated the possibility of obtaining inputs from various organizations. Thus, for the immediate support for research, the Swedish Organization SAREC is a likely contributor. For training 1986-87 in phytochemical methods for one candidate, the International Seminar in Chemistry, University of Uppsala, may be approached. For the experiemntal cultivation of medicinal plants, the Dutch Organization HIVOS would appear to be a logical source of financial support in view of its involvement in similar programmes in the country (vide extract in Annex 6). The expert's discussions enhanced this view. UNIDO's concern will logically be the provision of a pilot-scale unit for extraction formulation and packaging of the pharmaceuticals.

B. Research Capabilities in Chemistry Department, University of Botswana

The laboratories and equipment were a pleasant surprise for the expert. There are three research laboratories, one of which is hardly used. This particular laboratory, after some changes, is proposed by the expert to host for some years the new "Natural Products Research Laboratories". The details are given under the heading "Project Proposal".

C. The Present Progress in Cultivating and Post-harvest Treatment of Medicinal and Aromatic Plants

The expert has visited Kumakwane, where Thusano Lefatsheng has its nursery and experimental cultivation of medicinal and aromatic plants. The work is carried out by the horticulturist, Ian Martin. Some points of his experience so far are given in Annexes 2A and 2B.

Some kilometres further from Kumakwane, land (62 hectares) owned by Thusano Lefatsheng will be used for the enlarged activities. The electricity and water supplies seem to be sufficient - accessibility from the road is good (Annex 3).

D. Preliminary Market Research

The expert has studied very carefully all the market research made over the last 2 years by Thusano Lefatsheng. The intrest for and the desire to buy medicinal and aromatic plants and also essential oils is quite overwhelming. The situation can be summarized like this: There are no difficulties in finding customers; the problem for Thusano Lefatsheng is to cultivate enough quantities. Until now only one plant has been exported, namely, grapple, Harpagophytum Procumbens. The situation of today is that for the harvesting in 1986 Thusano Lefatsheng will get the grapple root from two sources:

(1) buy dried roots from collectors in the Kalahari region;
(2) harvest own plantations.

The dried root will be processed to Radix harpagophyti incisa. The price for this drug in 1985 was P7,25 per kg, whereas the production cost was P5,50 per kg (US $ = 2.10 Pula). The market for this plant is described in Annex 4.

E. Conclusion and Recommendation

All findings so far obtained are positive and therefore an integrated project is proposed by the expert after discussions with the appropriate authorities.

The constraint for development in Botswana is the lack of trained personnel. Therefore, the training component is very important.

3. Project Proposal

Based on the findings given in the previous paragraph, the expert has come to the conclusion that an integrated project with three main donors will best solve the problem. The Swedish Organization, SAREC, was contacted before the expert left Sweden (21 October 1985) and further discussions have tekan place in Gaborone. SAREC may finance most of the cost for the new laboratory; whereas the Dutch Organization HIVOS is interested in supporting most costs for the experimental cultivation. UNIDO is supposed to sponsor the extraction and packaging, the experts and one vehicle.

The integrated project proposal is shown in Scheme 1.

The indicative contribution from SAREC is given in Annex 5.

The likely contribution from HIVOS has yet to be determined, but within a major project designed to cultivate a variety of crops (see extract, Annex 6), cultivation of medicinal and aromatic plants may also be supported.

The contribution from UNIDO is proposed to be as follows:

I. Personnel		US$
(a) International Experts		
Pharmacologist/pharmacognosist	6 m/m	
Phytochemist	6 m/m	
Process technologist	2 m/m	
(b) Training		
Chemical technology	3 m/m	
Formulation, packaging	3 m/m	
Pharmacology	3 m/m	

II. Equipment		
1	Multipurpose pilot plant with extractor capacity 250 litres, complete for extraction, distillation, concentration of plant extracts	
1	Precooling unit (water from +25°C to +15°C).	
1	Grinding mill	
1	Machine for filling tea bags	
1	Machine for dispensing dried plant material in double film with printed text	
1	Printing machine for the film	
1	Machine for filling syrup	
1	Vehicle, 4-wheel drive for rough terrain	
	Chemicals, solvents, etc. for 3 years	10,000/year

The above integrated project proposal is considered as the phase where the existing tested raw material will be utilized to develop technology for processing. Alongside the development of technology, essential research nd development competence should be built up. For this purpose the Natural Products Research Laboratories is expected to get its own space in a new wing to be built in the University of Botswana. On the production side, it is foreseen that cultivation, extraction and manufacturing will be expanded to cover further plant species.

A. The Activity of the Natural Products Research Laboratory

When finally established, this laboratory will have the following functions:

(a) Integrated research on medicinal plants with the components ethnobotany and plant systematics, phyto-chemistry and pharamcology. It is now the intention that such studies would lead to higher degrees at the University of Botswana.

The selection of plants will be based on the inventory of traditional medicine carried on by Prof. Frantz Staugard (now in Sweden together with the botanist, Miss Helen Moss, B.Sc. The work will be based on the purification monitored by bioassay.

(b) Development of analytical techniques for the quality assessment of products manufactured by Thusano Lefatsheng. Generally, such chemical assay methods are developed by the application of results obtained from research. Accordingly, the laboratory will study the chemical nature of the plant extracts and formulation. Sometimes methods already developed could be adopted for this purpose.
For example, the expert has handed over the detailed description of a HPLC method for determination of the harpagoside content in the grapple root (Annex 7) which may be adopted as a method of quality control of products from this root.

B. Plant Species to be cultivated

The plant species proposed for cultivation are classified into various groups, as shown in Annex 8.

C. Extraction

The preparation of standardized extracts is necessary, both for consumption within the country and for export. It cannot be stressed enough how urgently a multipurpose exdtraction unit is needed.

As can be foreseen, this unit will be used also for production for several years; therefore the 250-litre model is proposed.

The pilot plant unit is expected to develop technology for processing essential oils, as there is a demand for perfumery substances both within Botswana as well as for export purposes.

D. Formulation and Packaging

This project will include the manufacturing of some simple galenical preparations like tea bags, strip-packed dried plants and syrups.

S C H E M E I

THE INTEGRATED SAREC-HIVOS-UNIDO PROJECT ON NATURAL PRODUCTS RESEARCH AND MANUFACTURING OF PLANT-DERIVED PHARMACEUTICALS

DURATION - 3 YEARS

Natural Products Research Laboratory, Department of Chemistry, U.B.	THUSANO LEFATSHENG		Formulation Packaging
	Experimental Cultivation	Extraction	
1. Integrated research on medicinal plants: ethnobotany, phytochemistry, pharmacology. Site: One of research laboratories, Dept. of Chemistry. Later on: own space. Co-ordinator: Dr. John Woollard with scientific assistance for pharmacology and botany Personnel: 2 Research Fellows, 1 Technician Training: Int. Chem. Sem. Uppsala and elsewhere Equipment: Existing and new Experts: Phytochemistry 6 m/m, Pharmacology 6 m/m, Process technologist 2 m/m Transport: Vehicle for plant collection and cultivation 2. Quality control of products and raw material from Thusano Lefatsheng	New buildings in the Kumakwane Region. Fencing Electricity supply Water supply Equipment for cultivation Training of personnel Vehicle	Equipment 1 multipurpose pilot plant extractor 1 250-litre pre-cooling unit (water from +25°C to 15°C) Grinding mill Solvents and chemicals	Equipment 1 machine for making tea bags 1 machine for including dry plant in double film sealed with heat 1 machine for filling syrup
FOLLOW UP Laboratory expansion	Large-scale cultivation	Large-scale extraction	Large-scale manufacturing

ANNEX 1

Visit to Botswana 7-20 December, 1985

7 Dec — Arrival; discussions with Dr. John Woollard, University of Botswana.

8 Dec — Discussions with Dr. Anne Sundbye and Dr. Carl Berg for collaboration with the proposed Natural Products Research Laboratory.

9 Dec

1. Meeting at UNDP office between the following persons:
   a. Mr. G. Bekele, SIDFA fom Lusaka. (He covers Zambia, Zimbabwe and Botswana)
   b. Mrs. K.S. Mugerwa, JPO in Gaborone.
   c. Dr. John Woollard, Dept. of Chemistry.
   d. Mrs. F. Horenburg, Thusano Lefatsheng.
   e. Professor Finn Sandberg, UNIDO expert.
2. Discussions at the University between Dr. Woollard, Mrs. Horenburg and the Expert, concerning the components of the project proposal.
3. Meeting with the Dean of Science, Dr. Ian Mac Fairlaine.
4. Detailed visit to all laboratories of the Department of Chemistry, U.B.

10 Dec

1. Visit to the Deputy Vice Chancellor of U.B. Professor Terence Davis.
2. Visit to Mr. Raymond Kwerepe, Ministry of Agriculture.
3. Visit to Dr. Rojas, Representative of WHO.
4. Visit to Department of Biology, U.B.
5. Visit to the office of Thusano Lefatsheng.

11 Dec	1. Visit to the Vice Chancellor of the University of Botswana, Professor T. Tlou. 2. Visit to the Permanent Secretary, Ministry of Health, Dr. D.B. Sebina. 3. Visit to Kumakwane; the nursery and land for the future extraction Unit of Thusano Lefatsheng; horticulturist Mr Ian Martin.
12 Dec	1. Visit to National Institute of Research: Mrs Ulla kann. 2. Visit to Mrs D.J. Tibone, Principal Industrial Officer, Ministry of Commerce and Industry 3. Visit to the Cheif Pharmacist. 4. Visit to Dr. Gus Nilsson, a Swedish Horticulturist of great experience.
13 Dec	1. Visit to Swedish Embassy to see Mr. A. Johnson who deals with Sarec projects. 2. Interview with Golline Madati, a candidate for future position as research fellow. 3. Interview with Motlapisa Seganabeng, also a candidate for future position as research fellow. 4. Discussion with the physiologist at University of Botswana, Miss T. Molethe.
14 Dec	Field trip (about 800 km) for prospection of land in the Tuli Block Estates along the Limpopo river (horticulturist Ian Martin, Mrs F. Horenburg and the expert and driver).
15 Dec	Meeting with Helen Moss, a Botanisk who had made all the collections for Dr Staugård.
16 Dec	1. Visit to Mr. Manathuko, Deputy Permanent Secretary, Ministry of Agriculture. 2. Visit to Mr Motseme, Cheif Agricultural Economist, Ministry of Agruculture.

3. Visit to Mr. Mothibatsele, Director of Industrial Affairs, Ministry of Commerce and Industry.

17 Dec

1. Visit to Mr Danam and Mr James, Botswana Power Corporation.
2. Visit to Mr. Afeta, Assistant director of Economic Affairs, Mr Matila, Planning Officer, Ministry of Finance.
3. Discussion with Chemistry department.

18 Dec

1. Discussion between Ulla Kann, Dr.McFairlane Dr. J. Woollard, Mrs F. Horenburg, Prof F. Sandberg.
2. Visit to K.L. Verma, Kgalagadi Soap Industry.

19 Dec

1. Visit to A. Campbell, Director Museum
2. Visti to M. Parkinson, Cheif Agricultural officer (B.D.C.)
3. Mrs Ektvedt (NHI) Pharmacy tutor and Mrs Manyeneng (counterpart)
4. Visit to Mr Von Spoeneck, UNDP Resident Representative.
5. Interview with Mr Sekolo ,
6. Ulla Kann

20 Dec

1. Meeting with V.C. Prof. Thlo (UB), Dr McFairlane, Mrs U. Kann, F. Horenburg, Dr J. Woollard, Prof Sandberg.
2. Visit to UNDP.

ANNEX 2A

Observations On Harpagophytum Procumbens And H. Zoyheri

The two species of Harpagophytum native to Botswana have been grown at Kumakwane Gardens for Thusano Lefatsheng research project for the past 2 years. Seed of H. procumbens was supplied by Dr. Tietema of NIR, and was obtained from the sanveld of the Kalahari. Seed of H. Zoyheri was obtained from wild plants growing in the vicinity of Kumakwane. This latter species is considered an inhabitant of "Hardveld" although the areas where it grows around Kumakwane are docse, coarse sand of at least one metre and normally around 3 metres in dept. When the two species are grown together there has been no consistent difference between the plants (leaves, flowers or fruit) so it is open to question whether they are indeed two species.

The first sowing was of only 10 seeds, made in February 1984. This resulted in 4 plants of which two subsequently died from caterpillar damage to the stems of the seedlings. The plant flowered in the few months of the hot season remaining, and in fact did not die down until the cold season was almost over, late July, and probably from the soil drying up as there had been no severe frost. As the weather became warmer again in September (1984) and with the first rains, shoots appeared again. This year was to prove bad for infestations of red spider mite (Tetranychus cinnabarinus) both in the garden and on surrounding wild plants in the vicity of Kumakwane, and the Harpagophytum was one of the many species to be damaged by the pest. It is not known whether this did or can affect the yields of the tubers.

In November 29 and January 10 (1985) seeds of Harpagophytum zeyheri were sown. Seed was sown both with and without the woody pericarp, but no appreciable difference was found in germination when it is taken into consideration that 35% of the woody "seeds" contained no seeds at all. Germination was enhanced by a period of wet, overcast wether but only averaged 20%. This figure was further reduced by the Multitude of pests that found the germinating seedlings to their taste. These insects included larvae of ground beetle and cutworm. The pests are also very attrative food for a wide range of insects such as grasshoppers, crickets and dusty brown beetles; more mature plants they disfigure the leaves and may affect yields. The result of this poor germination and depredation by insects was a stand of around 5% of the number of seed sown.

These sowings received no water once the seedlings had germinated. The rainfall in the rainy season August 1984 to April 1985 was around 130mm, most of which fell before the end of November. The plants thus grew under condition of minimal water. And with no pest control there was considerable leaf damage. There was the benefit of 40% shadecloth plus a soil which had had organic matter added to it over the previous 3 years.

The plants were dug up by members of the National Institute of Research and the tubers weighed in July 1985 when the top growth had still not entirely died back (this cool season was again without frost of any consequence). N.I.R. have the figures of the weights of combined parent and storage tubers, I believe they were around 400g. This is considered well above average when compared to naturally growing plants.

The storage tubers were removed for analysis, and the parent tubers were replanted in August 1985, in sequence where the first harvest yield can be compared to subsequent performance of the parent tubers. The amount of shoot growth is already at the date of writing equivalent to the final growth in the first season, and so far this year prest damage to the leaves has been minimal (but some tubers were attacked by cutworm). Flowering is profuse, but as in the first season the percentage of flowers to set fruit is low, less than 10%.

Rainfall this year is very poor, about 35mm todate, so two irrigations have been given. More seeds are being sown, in seedtrays this time to try and eliminate seedling losses.

It is difficult to extrapolate to give a reliable estimate of yield, but is appears that in deep fertile sandy soils at least 2kg/m^2 (equivalent to 20 tons/hectare) would be possible from complete harvesting of a one year crop. In the wild only the superficial storage tubers are harvested, to enable the plant to regenerate the following season in condition of natural rainfall only, but it is likely that with even limited amounts of water for irrigation complete harvesting and replanting of the parent tuber is appropriate. This method of total harvesting is somewhat akin to the manner of growing the potato, Solunum tuberosum; it is not known whether vegetative propagation from cuttings like the method of propagating sweet potato is a practical alternative or supplement to increase numbers of plants.

J. A. Martin, 17 December 1985

ANNEX 2B

List of Aromatic and Medicinal Plants Grown at Kumakwane, Botswana

SPECIES	COMMENTS ON GROWING
Achillea millefolium	Grows easily with irrigation but reluctant to flower.
Adonsonia digitata and A. gregorii	Reasonably easy to grow except for risk from frost and slow growth.
Agastache rugosa	Grows easily with irrigation if nematoes are controlled.
Agave americana and A.sp.	Easy to grow with little or no irrigation.
Allium schoenopraesum	Easy to grow with irrigation.
Aloe vera	Needs some protection from hot dry climate and cutdown.
Althea rosea var nigra	Possible to grow, with irrigation, shade and spider mite control.
Amarathus spp (wide range of indigenous and exotic species)	Easy to grow, especially with irrigation
Argania spinosa	Difficult to germinate, otherwise easy to grow.
Argemone mexicana	Easy to grow although seedings can be killed by overwatering.
Arnica montana	Suprisingly easy to germinate and can be grown in moist, shaded area.
Artemesia afra	Easy to grow, even without irrigation.
Ascelpias fruticosa	Easy to grow.
Atriplex spp.	Easy to grow.
Balanites aegyptiaca	Easy to grow.
Bauhinia petersiana, B. macrantha	Not easy to germinate, otherwise easy to grow.
Bixa orellana	Susceptible to seeding diseases, otherwise easy to grow where ppest is not severe.
Blumoa galpinii	Difficult to germinate, otherwise very easy to grow without irrigation.
Boophane disticha	Easy to grow.

Calendula officinalis	Susceptible to a wide range of pests and diseases especially in the hot season. More suited to cool season production.
Cajanus cajan	Easy to grow. May be attacked by spider mite.
Calotropis procera	Easy to grow.
Capsicum spp.	Easy to grow provided nematoes and aphids are controlled.
Carbenia benedicta	Grown best under shade with fertile soil and irrigation. Susceptible to leaf-eating caterpillars.
Cassia acutifolia, C. angustifolia	Does not require special conditions but susceptible to spider mite and Cercospora sp.
Catharanthus roseus	Easy to grow.
Centaurium erythraea	Not suited to this climate.
Chenopodium ambrosoides	Difficult to germinate.
Chrysanthemum cineraefolium	Can be grown with irrigation but slow to flower.
C. parthenium	Grows easily but to produce high quality leaves takes close attention to watering and nutrition.
Cicar arietum	Easy to grow into cool season if nematodes are controlled.
Coriandrum sativum	Needs special conditions to grow and seed well in this climate.
Cucurbita foetidissima	Easy to grow, even without irrigation, but performance may be adversely affected by nematodes, spider mite and aphids if these are not controlled.
Cynara Scolymus	Easy to grow under shade netting, with irrigation. Occasional attacks from leafeating caterpillars.
Datura innoxia	Very easy to grow, lasts for at least 2 years.
D. stramonium	Very easy to grow, but seeds scatter readily so becoming a weed.
Digitalis lanata	Easy to grow if scale insect controlled.
Echinacea (Rudbekia)	Needs irrigation and protection from extremes of sun and wind.

Eryrhrina spp.	Not easy to germinate, otherwise easy to grow where pest is not severe.
Eryngium maritimum	Not easy to grow.
Escholtzia californica	Easy to grow.
Foeniculum vulgare	Prefers shade and needs control of aphids and spider mite.
Grewia spp.	Easy to grow.
Gypsophila paniculata	Difficult to transplant, but otherwise very easy to grow and able to survive above.
Glycerrhiza glabra	Difficult to germinate.
Harpagophytum procumbens H. Zeyheri	Se separate notes.
Hibiscus sabdariffa	Robust plant, easy to grow under limited irrigation provided virus--transmitting spids are controlled.
Hypericum perforatum	Require irrigation, fertile soil and shade.
Hyoscyamus niger	Easy to grow, at least under nethouse conditions.
Hyssopus officinalis	Easy to grow with limited irrigation provided mites and aphids are controlled.
Lablav purpureus	Easy to grow.
Lavandula angustifolia	Hardy to condition of climate in Botswana but susceptible to a number of pests and diseases.
L. stoechas	Less susceptible to pests that the above species.
Leptospermum citratrum	Difficult to transplant as a seedling, otherwise easy to grow as it apparently has no pests or diseases.
Lobelia inflata	Difficult to grow in this climate.
Luffa cylindrica	Prefers a rich soil. Grows well if aphids and spider mite are controlled. Only flowers in short days.
Marianum silybum	Not easy to grow in this climate.
Malva glabra	Needs protected site and watering to grow well.

Matricaria recutita	Best grown in cool season. Very susceptible to powdery nilden.
Mentha x piperita	Needs shade, good soil and lots of water and occasional pest and disease control. If all these conditions are met it yields very well.
Monarda didyma	Can be grown but needs careful management in this climate in order to produce quality leaves.
Ocimum americanum	Easy to grow but benefits from good soil and watering. The easiest of the species to grow.
O. canum	As O. americanum.
Oenethera biennis	Some aspect of its requirements has yet to be resolved as it does not prosper. Plant very susceptible to over watering.
O. glazonana	Somewhat easier to grow in this climate.
Ononis spinosa	Not easy to grow in this climate.
Oreganum majorana, also O. vulgare	Easy to grow with irrigation of spider mite controlled.
Papaver somnifera	Easy to grow in cool season provided spider mite controlled.
Phaseolus acutifolius	Easy to grow even with minimal watering provided spider mite controlled.
Pimpinella anisum	Not easy to grow in this climate.
Plantago lanceolatum	Easy to grow given some shading and watering.
Plumvago zeyheri	Easy to grow. Native plant.
Potentilla erecta	Needs shading and watering.
Pterodiscus speciosus	Easy to grow. Native medicinal plant.
Ricinus communis	Easy to grow. Naturalized plant.
Ricinodendron rautenii	Easy to grow where frost is not severe. The seeds are very hard and need treatment.
Rosmannus officinalis	Not easy to germinate of transplant, but otherwise grows well with shade netting and limited watering.

Salvia officinalis	Grows well provided pests and diseases are controlled.
S. sclarea	Needs high level of management.
Sanuisorbia minor	Fairly easy to grow.
Sanseveria aethiopica	Easy to grow.
Saponaria officinalis	Very tough plant due to extensive root system and has few pests.
Satureja hortenis	Grows well in fertile soil with watering.
Sclerocarya caffra	Easy to grow. Indigenous tree.
Sesamum triphyllum	Easy to grow. Indigenous herb.
Sesbania sesbans	Easy to grow. Indigenous herb.
Simmondsia chinensis	Slow to establish but otherwise trouble-free.
Sinapis alba	Very susceptible to pests (bragrada bug)
Solanum laciniatum	Fairly easy to grow.
Strychnos cocculoides	Difficult to transplant. Otherwise very easy yo grow. Indigenous tree.
Symphytum x uplandicum	Grows easily in rich moist soil but difficult to grow from seed.
Tagetes patula	Easy to grow but susceptible to spider mite.
Thymus vulgaris	Not easy to transplant older plants, otherwise easy to grow with minimal watering.
Trigonella foenum-graecum	Easy to grow in the cool season.
Tylosema esculenmtum	Easy to grow. Susceptible to spider mite.
Valeriana officinalis	Easy to grow in shade house.
Verbena citratum	Easy to grow.
Viola tricolor	Grown in shade house with irrigation.
Vigna tribola	Grows easily. native climber.
Ximenia americana and X. caffra	No success with germination yet. Indigenous shrub.
Zisyphus mauritanica, Z. mucronata, Z. spina-cristi	Easy to grow.

ANNEX 3

The Activities of Thusano Lefatsheng
In the Kumakwane Region

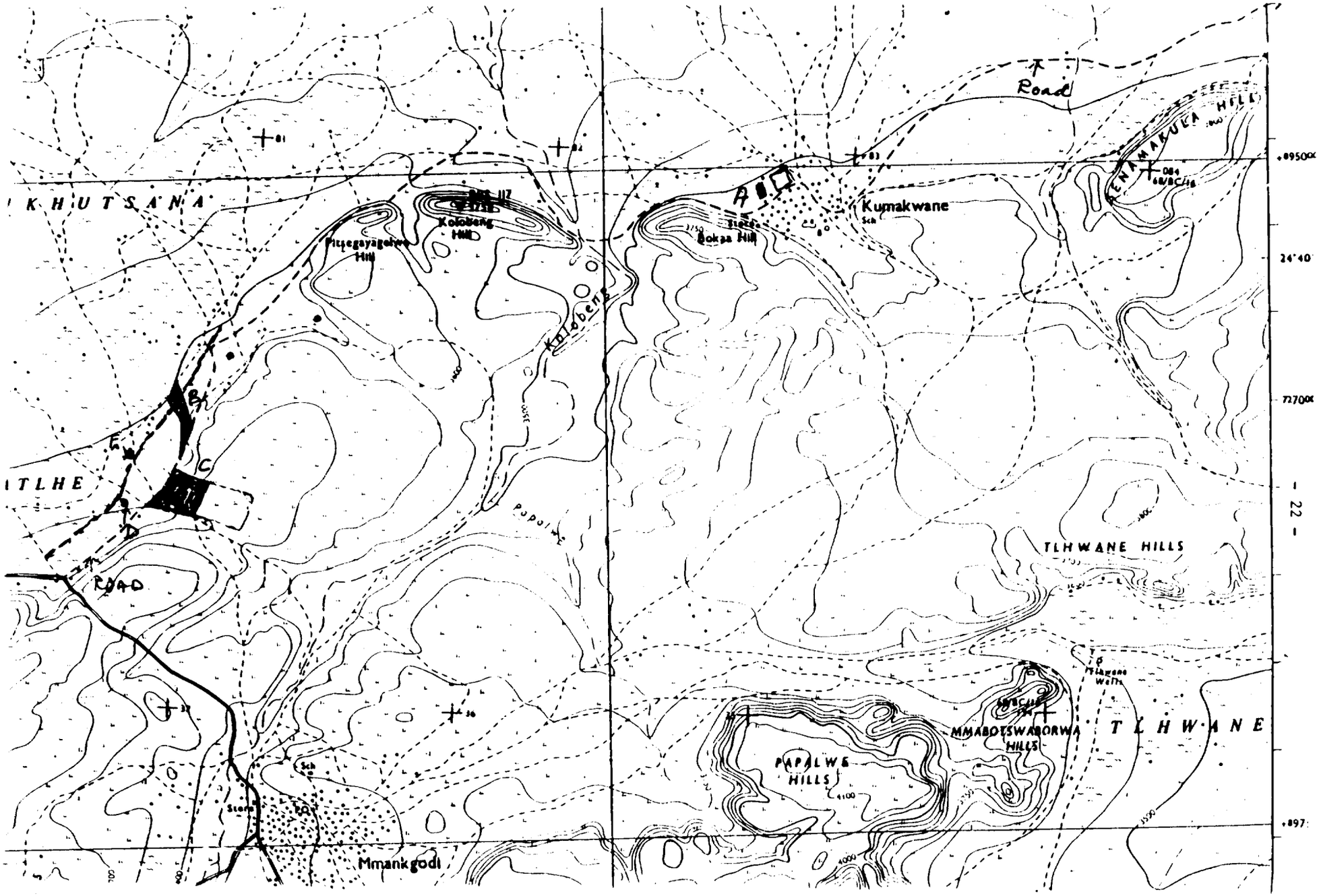
A. Nursery (with and without black net).

B. Land for cultivation of medicinal plants.

C. Land for buildings (pilot plant extraction and packaging) and for cultivation of medicinal plants.

D. Borehole: Dept 45m. with pumping rate 60m^3/hour even after 72 hours (height 26,00m - from starting at 16,70m). Recovery was 14.10m after 8 hours and 13.14m after 24 hours).

E. Electric Power Supply, from which a transformator will be installed and a cable drawn to C.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KENAMABULA HILLS
Kumakwane
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Bokaa Hill
Kolobeng Hill
Pitsegayageleve Hill
Kolobeng
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P.O.
PAPALWE HILLS
MMABOTSWARORWA HILLS
TLHWANE HILLS
T L H W A N E
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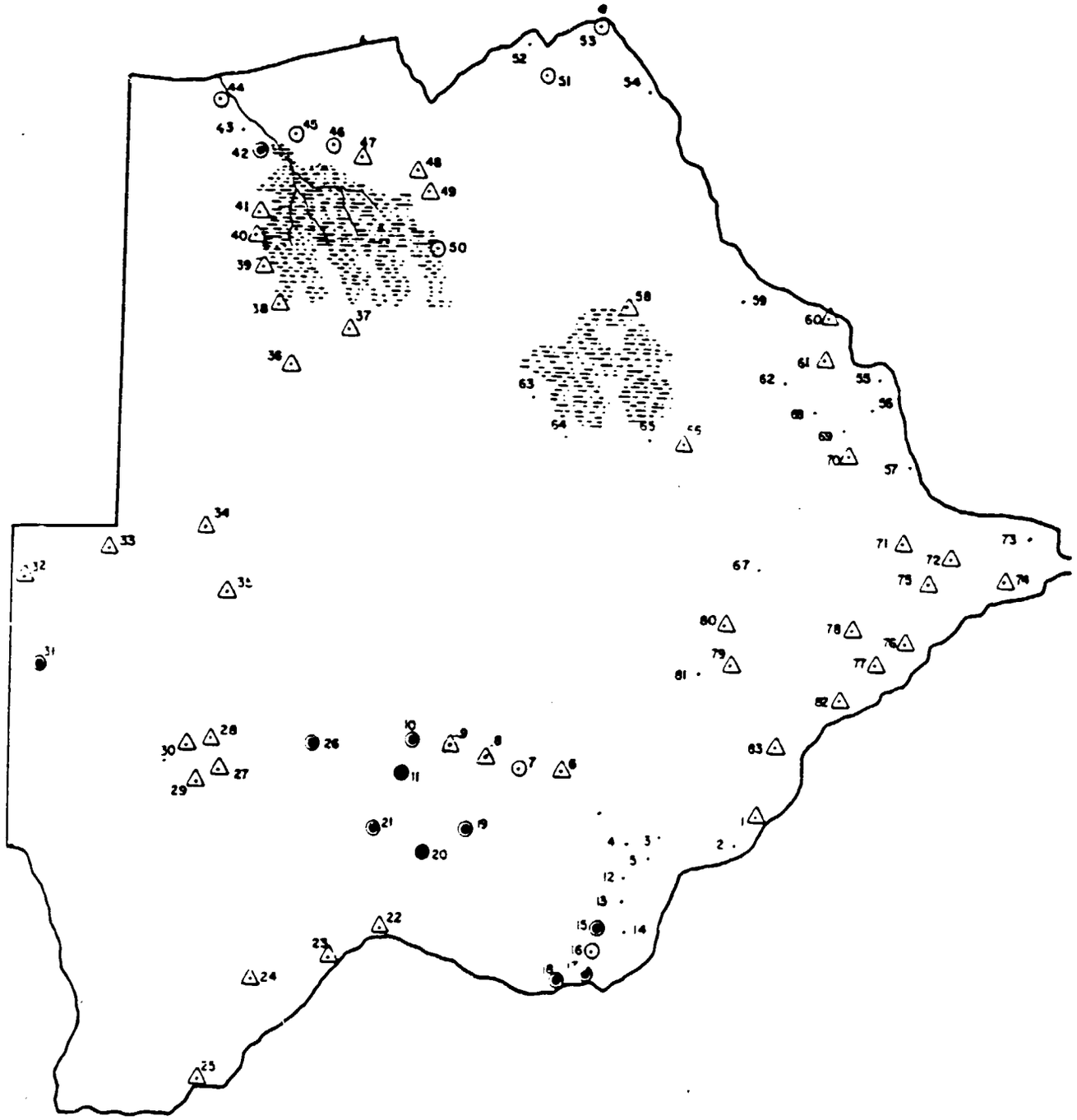
DENSITY & DISTRIBUTION of *Harpagophytum procumbens*



Symbol	Density	Rating Scale approx. No. plants per 20 000m²
●	High	50 +
◍	Medium	5 - 49
○	Low	1 - 4
△	Unreliable data	

ANNEX 4

GRAPPLE

OTHER NAMES

Harpagophytum procumbens, Sengaparile, Devil's Claw.

THE RESOURCE

The Roots

The harvestable portion of the plant is the set of secondary storage roots which radiate out from the vertical primary root. These secondary roots are spherical to ovoid in shape, measuring up to 12 cm in diameter, and weighing up to 4 kg.

Distribution

Grapple occurs mostly on red Kalahari sand, but also shows a tolerance for calcrete soils. It occurs throughout Botswana, mostly in the Kalahari, and often on disturbed sites, (se enclosed map).

Harvest Period

1 April to 31 October (by legislation).

USES

Traditional Uses

The secondary roots are used as a medicine to "clean the blood", stomach disorders, etc.

Proposed Use

Export of raw and processed Grapple.

BUYING

Method

Briefly, it is proposed that buying day be officially organised every six weeks in each village where the harvesting is undertaken. The dried roots would be inspected by a government officer to ensure that no primary roots were present, and then the buyers would offer their prices to the diggers. Thus the diggers should receive competitive offers.

It is proposed that the buying price be approximately P2.50 per kg.

Problems

The main problems probably will be: drought; ensuring the timely issue of Extraction Permits to the diggers and ensuring that the buying actually does take place on the pre-arranged days.

PROCESSING

Processing of dried Grapple roots should only be considered if the final product is to be prepacked for the market. All major overseas importers are either raw drug distributors or manufacturers, and as a general rule they will only import whole dried slices of Grapple as they are afraid of adulteration, which is not unknown in the trade.

Fortunately, the active ingredients in Grapple are sufficiently concentrated so as not to require any special refining. Thus, most of the Grapple is sold in tea form, i.e. as a powder, granulated, or as tablets and capsules. Only two major pharmaceutical firms were found to produce extracts, one in liquid form to be drunk with water (Bio-Botanica, Farmingdale, New York) and the other in a phial for injection (E. Hagen, Freilassing, W. Germany).

The most important process is sterilisation of the Grapple to ensure that it has no bacterial contamination. (One Namibian exporter distributed thousands of pre-packed Grapple to hundreds of German stores and all had to be recalled when German inspectors found E. coli contamination, causing irreparable harm to his business besides giving the medicine a bad name).

Sterilisation

The usual method of sterilisation is fumigation. It is important to use an internationally recognised method.

Granulated Roots

The root slices are coarse-ground (Radix harpagophylincisa) and usually packed in 100g retail packs to be used for tea. Sometimes the granules are packed in tea sachets, 100g to a box.

Powdered Roots

The root slices are ground to a fine powder which can then be put into capsules or made into tablets.

Extracts

The extraction will take place as soon as the pilot plant unit (CR611) is installed.

Assay

For 1986 production, quality control will be introduced; the content of Harpagorid will be given (determined by HPLC-method).

PACKAGING

Bulk Export

In this case the normal method is to pack the dried root slices in heavy duty plastic bags of a standard weight, sealed, and then sewn into hessian bags. The reason for the plastic bag is to ensure that no moisture gets into the dried root slices on the journey as the roots are hygroscopic, i.e. they attact and absorb moisture. If they become damp they go mouldy and/or turn black.

Consumer Packaging

The design of this package needs to be done professionally as it is important to convey the image of a quality product by a first-rate organisation. The logo and layout is very important. The printing and fabrication of the box also must be top quality.

SHELF LIFE

Tests show that after 5 years there should be no deterioration in quality (Scheffler, pers. comm; 1981).

MARKETS

Controls

Most countries are not concerned about imports of raw medicinal herbs, provided they are not dangerous. Raw medicinal herbs may be imported in the crude dry state (dried roots, leaves, bark, etc.) or in granulated or powdered forms.

If the drug is not registered then only the name and dosage instructions (no medical claims) may be put on containers of pre-packaged tablets, capsules, etc.

The registration of drugs is a costly and time-consuming operation. The laboratory and clinical trials have to be carried out under the supervision of a Government medical department, the costs being borne by the applicant which is usually private enterprise. The result is that no many herbal drugs are registered. Only big companies can afford to do it, and will only do so if they have sufficient control over the resource so as not to have all the small drug companies swamp the market.

Some companies ignore the ruling and market unregistered drugs with medical claims printed on the packages, hoping that by the time an inspector discovers the ploy, the product is well established on the market.

Perhaps the most subtle way around this problem is to call the Grapple "Arthritis Root", and to sell it under that trade name. Thus no regulations concerning advertising claims are being broken.

In most countries visited on the market survey, the only legal way to advertise medical claims of unregistered drugs in shops, is to provide literature, though this literature may not be placed next to the products in question.

Grapple has been registered in Germany and France, and according to information received, it is presently under consideration in the UK and Belgium.

Importing Countries

The main importing country is Germany, which probably imports 60 - 70% of the resource. Most of this comes from Namibia and probably totals about 80 tonnes per year. The German companies either re-export in the raw state, or process and then distribute it. The following countries, besides Germany are known to import raw Grapple: France, Holland, Belgium, Switzerland, UK, Portugal, Spain, Canada, and USA.

Prices - Unprocessed

It has proved impossible to obtain firm export prices from Namibia but the following prices have been obtained:

- a Namibian Exporter, S.W.A. Teufelskralle (Pty) Ltd., have offered to pay up to P4 per kg for Grapple delivered to Windhoek.
- a South African Exporter, Kruger & B' ty) Ltd., have offered to pay up to P4.50 per kg for Grapple FOB one.
- German Importers claimed they had been offered Grapple at US$4,00 (P4,38) per kg in 1982, but evidently were determined to bargain the price down to $3,50 (P3,83) per kg. These same importes were wholesaling the unprocessed root at DM15 (P6,80) per kg.
- U.S. Importers In 1981 one U.S. importer obtained supplies from a Cape Town exporter (Kruger & Behr) at $4,50 (P4,93) per kg c&f New York. The same importer claimed that in 1982 the price dropped to $4,00 (P4,38) per kg. (At the end of 1982 the same S.A. exporter was offering to buy Grapple from VPR at P4,50 per kg f.o.r. Gaborone).

From the above it will be realised that either the Grapple market flucturates considerably or else there is a considerable amount of misinformation being given out. The only hard facts are that S.W.A. Teufelskralle (Pty) Ltd., of Windhoek will pay P4,50 per kg. delivered Windhoek, and Kruger & Behr of Cape Town will pay P4,50 f.o.r. Gaborone. The Namibian buyer has the potential to purchase larger quantities.

Comparative Wholesale Prices for Processed Grapple

Germany:	
powder in bulk, per kg	DM15 = P6,80 per kg
U.S.A.:	
100 x 510 mg capsules in bulk	$2,50 = P54,75 per kg
Powder in bulk, per kg	$7,26 = P7,95 per kg
R.S.A.:	
100 x 400mg tablets in bottle	R2,31 = P57,75 per kg
100g powder in a box	R2,25 = P22,50 per kg

Note: i. The bulk prices of powder are the prices charged to manufacturers.

ii. Gelatine capsules in which the powder is placed cost about 1 thebe each.

iii. It is not known how much it costs to make tables.

Estimate of Market Potential

The market potential has been very hard to estimate. S.W.A. Teufelskralle (Pty) Ltd indicated that they may like to import from Botswana as much as 30 tonnes per year "if the price is right" (i.e. significantly less than R4,50 per kg landed Windhoek).

From the number of enquiries received from European and South African importers, it probably would be possible to export a further 10 tonnes. This market should be sufficient for the Pilot Project.

If the drug can be registered in the U.S., then the demand will completely outstrip the supply, almost certainly causing an increase in the price.

Promotion and Marketing

From discussions with distributors in Germany it is clear that the lack of advertising on Grapple has severely affected the sale of the drug.

The best promotion that can be given to the marketing is twofold:

- Registration of the drug in the U.S. in particular.
- High exposure in health magazines and other media, especially in magazines aimed at an elderly readership.

The design and quality of the containers for pre-packaging, is of prime importance.

A joint venture or marketing arrangement with a major European pharmacetical firm could be very advantageous. Unfortunately, the manufacture and packaging of an important drug by a developing country is not conducive to consumer appeal in Europe, so using the brand name of a well-established European pharmaceutical firm could be of immense value. E. Hagen is prepared both to assist in registering the drug in the U.S. and in marketing.

Competition

Namibia is a major competitor. There are indications that their resource is dwindling due to lack of controls, so with careful husbandry the Botswana resource ultimately could control the world market.

Every year scores of new drugs are launched on the U.S. market. Most of them are variations on an existing medicine; the greatest distinction of the newcomer is a catchier name. Of the 96 drugs approved in 1981, only three were judged by the FDA to represent important therapeutic gains.

A considerable amount of money is put into splashy advertising campaigns on arthritis medication, often with spectacular results; for example, Oraflex was given a major advertising promotion and US$6 million in sales were chalked up in the first month. However, it is important that advertising be conservative in its claims for therapeutic values, as overblown claims can cause the product's downfall.

What is clear, however, is that a well planned promotion of an arthritic drug in the U.S. can pay dividends. There is no reason why this should not be the case elsewhere in the world, as arthritis is a world-wide problem.

All clinical and research evidence concerning Grapple shows that it is effective with over 60% (sometimes 100%) of arthritic patients in clinical trials and no harmful side effects have been identified.

COSTING

Capital Requirements for Pilot Project

The following building space and equipment may be shared with those required for other projects:

- Satellite Buying Centres.
- Storage space for raw and finished products.
- Transport.

Special equipment required for this project will include:

- grinding and powdering machines
- tablet machine
- capsule filling machine.

A special area will be required to process the Grapple.

Gross Profit Calculations

Only the gross profit on unprocessed Grapple will be considered here as the processing costs to produce tablets and capsules are not known. It would seem evident that processing is more profitable but only further research will reveal by how much.

Wholesale price:		
Raw Grapple per kg f.o.b. Gaborone		P4,00
Less Direct Costs:		
Cost of 1 kg Grapple	P2,50	
Cost of purchase (20%)	,50	
Cost of packing	,10	
Misc	,15	3,25
Gross Profit		,75

Gross Profit Percentage = 23%

EMPLOYMENT AND INCOME FACTORS

Present and Potential Employment

Taylor (1981) noted that about 600 people were engaged annually in the digging of Grapple. Once the management of the resource is under control the number of people involved could run into several thousands.

Present Income

In a good season, i.e. no drought, some people earn over P200.

Income Potential

With competitive buying, and the higher floor price, the diggers should earn 25 to 50% more.

ANNEX 5

INDICATIVE CONTRIBUTION FROM SAREC FOR THE
Integrated SAREC-HIVOS-UNIDO project
in Botswana

1. EQUIPMENT (Laboratory equipment for quality control of products and raw materials)

A. For pharmacological research

Equipment for isolated organ	SEK
Polygraph, Model 79D + transducer	71000
Electrical stimulator	9675
circulation pump (HETO, Ninolab)	4750
Organbath (2)	550
Tubes, flashes etc.	770

B. For phytochemical research

Freeze-dryer, 4 kg, Edwards	36000
Mill (type SKI, Retsch) with devices	11400
Stirrer for 40 liters (Werner-Glas)	3300
Fraction collector, Midifrac.	7150
Rapid fraction collector for flush chromatography:	
Gilson-pump, model 302	15900
Head 100C	5875
Puls damper 803C	11500
Fraction collector 201	21800
HPLC-device	
columns 10-ODS2-24314	8457
LDC-pump Constametic III	32600
LDC-spectromonitor 3000	42300
Rheodyne injector 7125	4900
Rotation-evaporator 10L81-50 (Werner-Glas)	33000
Flush-columns (Werner-Glas) No. 148-60	455
Stainless-steel flash 10 liter + 20 liter	1245
TLC-plates:	
Kiselgel 60 1 kg (70-230 mesh)	695
Al-oxid 90 1 kg (neutral)	300
Plates:	
Kiselgel 60 (20 x 20, 5 x 20) 512 + 435	950
Al-oxid 60F (20 x 20, 5 x 20) 345 + 650	995
For preparation of plates	
Desaga, standard equipment (120,300)	16800
" . preparation device (120,305)	10425
Transp.	352.792

	Transp:	352792
	Lobar-columns	
	Ready made 310 mm RP8	3975
	" " 4/125 mm RP18	13000
	" " 4/250 mm RP18	14000
	UV-box - Cabinet II	6400
2.	LITERATURE	2500
3.	EXPERT SERVICES	
	Project technical adviser, 3 x 1 m/m	?
4.	TRAINING	
	Research fellowships, 12 m (2 x 6 m)	?
	Technician training, 6 m	?
5.	SPECIAL SERVICES	
	Cost of special analytical services at Swedish institutions	20000/year
	Estimated total contribution	412667

V FINANCIAL AND ECONOMIC ANALYSIS ANNEX 6

75. The Project funds will be used (a) to pay the capital costs of establishing the Thusano Lefatsheng Centre; (b) to cover the recurrent deficit on the Centre's operations from 1986 to 1992; and (c) to provide a revolving loan fund together with demonstration and extension services to assist at least 45 outgrowers to establish their own cash-crop growing enterprises by 1992.

76. The estimated capital costs of Phase I, 1986-89 and Phase II, 1989-92 are set out in detail in Tables 1 and 3 below. Tables 2 and 4 contain notional breakdowns of these capital requests by main budget category ; thus in Phase I about 22 per cent of the capital will be expended on purposes broadly described as "Administration", 22% on "Extension.Research,Demonstration", and 56% on "Commercial" facilities; of the much smaller amount of capital in Phase II, seven percent is for Administration, 32% for Extension, etc and 61% for Commercial purposes.

77. Estimated recurrent costs for Phase I are set out in Table 5 and for Phase II in Table 7. These estimates have been broken down by budget category in Tables 6 and 8 ; the breakdowns indicate that in Phase I some 31% of recurrent expenditure will be on Administration, 22% on Extension, etc, and 47% on Commercial operations; these proportions are virtually unchanged in Phase II (32%,20%, 48%).

78. The estimates in this memorandum are based on December 1985 quoted prices,salaries and wage levels. An allowance for 15% has been made for contingencies including inflation (for discussion, see paragraph below). Depreciation and replacement of assets have been handled by the sinking fund method,i.e. treated as cash costs, not below-the-line items.

79. The estimated gross revenues (i.e. total cash income) of the project for each of the seven years from 1986/87 to 1992/93 are set out in table 9. The estimates are considered to be cautious in term of potential gross returns from the various high-value crops which it is planned to grow at the centre itself. They are much less conservative in respect of the returns which are expected to accrue to the centre from marketing crops on behalf of outgrowers (whose total area under cultivation and total production is planned to greatly exceed that of the centre itself from 1990 onward).

80. The revenue estimates are subject to a wide margin of error. Many of the crops to be grown are new to Botswana, with high risks of crop failure.There is also some uncertainty about market conditions and opportunities: prices of some high-value crops can vary widely from one season to another as a result of gluts and shortages in other growing areas and markets at the other side of the world.

81. A flexible and opportunistic approach to the annual selection of crops to be grown is indicated. First, research must establish which plants can be successfully grown in Botswana field conditions, including the less than ideal circumstances under which the outgrowers work. Even then, the decisions as to which of the various crops to plant in any one season are best taken at the last moment in the light of the latest available information about market conditions. In these conditions it would be of only academic interest to make forecasts for years ahead of the exotic crops to be planted, yields, and prices to be obtained in order to justify the gross revenue estimates contained in Table 9.

82. The Thusano Lefatsheng Centre's own production on 10 hectares in the first column of Table 9 has therefore been estimated by using an average of the known yields and gross cash returns per hectare already being obtained in Botswana under low-technology irrigated conditions. Typical yields and cash returns per hectare in 1985 were: maize, 6 tonnes/ha @ P 224 per ton = P 1 344 per hectare; carrots, 20t/ha @ P 300/t = P 6 000/ha ; oranges, 25t/ha @ P 175/t = P 4375/t ; cabbages, 30t/ha @ P 200/t = P 6 000/ha.

83. These figures are for production on land which has been worked for some years unlike that at the centre which is still being cleared and will at first be less productive. Nevertheless, the cautious nature of the revenue estimates will be obvious, since at the end of the third (1988/89) cropping season the gross income from 10 hectares is estimated at only P 40 000 as compared with the notional P 60 000 obtainable from growing carrots or cabbages; a gross income of P 60 000 is only projected for the fifth (1990/91) season.

84. Revenues from tree crops on the 50 hectares hillside unit (column 2, Table 9) have also been cautiously estimated. Only 25 hectares of the hillside can be planted; the rest either has too steep a slope, is needed for water catchment purposes, or is

virtually solid rock. A typical yield of fresh grapes under low-technology in Botswana conditions could be two tons per hectare, selling wholesale at P 850 per ton. Thus, if the entire 25 hectares of available hillside were to be planted to grapes in 1986/87, crops of 50 tons worth P 42 500 would in theory be obtainable from 1989/90 onward. The projections in Table 9 show gross income from tree crops of only P 10 000 in 1989/90, rising to P 30 000 in 1992/93.

85. Estimated gross returns from the centre's 20% margin on sales of gathered wild produce (column 3, Table 9) have also been kept far below the potential returns which it is believed are obtainable from this source. The estimates are based on projected returns from such low-value wild products as florists' decorative grasses, tree twigs, and traditional building materials (poles, thatch, etc). No account has been taken of the potential yields (both to the centre and the gatherers) from the variety of medicinal, herbal and aromatic wild plants which were under investigation in December 1985.

86. Estimated revenues from the centre's 20% margin on sales of outgrowers'produce (column 4, Table 9) have been calculated on a more precise arithmetical basis than the other revenue estimates. This is because detailed studies of three crops (chillies, tobacco, morogo) are already available. The figures used in Table 10 have been drawn from the 1984 product profiles which are attached at Appendix II. The figures showing 1984 estimated income from Thusano Lefatsheng's 20% margin on outgrowers' sales have been converted into 1985 pula and included in Table 9.

87. It should be stressed, however, that although the calculations in Table 10 (as transferred to Table 9) are based on the very low crop forecasts in the product profiles, no allowance has been made in these calculations for the possibilities of either a complete crop failure or a collapse of the market for any of the three products. A further element of uncertainty (and possible over-estimation) arises from the crucial assumption that the projected numbers of outgrowers (rising from five in 1987/88 to 45 in 1991/92) can be recruited on schedule, with no dropouts.

88. Thus, while the gross revenue forecasts in Table 9 in respect of the centre's own production and of gathered wild products may turn out to be underestimates, the figures for outgrower production

(which ought to bring in 36 per cent of the centre's total gross revenues in the period 1986/92) represent an extremely demanding set of annual targets. It is recognised that these targets can only be met under the most favourable conditions and by devoting a high proportion of Thusano Lefatsheng's management and technical manpower to demonstration and extension activities, if necessary at the cost of neglecting some of the centre's direct revenue-producing operations.

89. Given the centre's primary aim of increasing incomes of subsistence farmers in the project area, the prime importance of meeting Thusano Lefatsheng's revenue target from its 20% margin in respect of gathered products and outgrowers'production can be seen from Tables 9 and 10. If the centre makes its target gross income of P 63 000 from its 20% margin on gathered products in the seven-year period 1986/93, the gatherers will receive P 252 000 for their work. If the centre makes its gross income target of P 327 500 from outgrowers' produce in the same period, then 60 outgrower households or about 420 people will receive net incomes totalling P 480 000, or roughly P 1 000 a year per worker as compared with the government minimum wage of about P 1 200 a year.

90. It will be evident from the explanations and analysis in paragraphs 79 to 89 above that this is a high-risk project where even indicative revenue estimates contain a wide margin of error. Nevertheless the project management believes on the basis of experience and in the light of prevailing economic conditions in Southern Africa (see paragraph below) that the gross income targets set out in this memorandum can be met within the 1986/92 project period.

91. By extracting figures from Tables 5 to 9, it is possible to calculate the percentages of the three main budgetary categories of recurrent costs which can be met from the projected revenues in Phase I, in Phase II, and in the sixth (1991/92) year of the project. These calculations are below:

(see page 27)

92.

Period	Administr. costs	Extension, Research, etc, costs	Commercial Operations costs	Total Costs	Gros Revenue
(a)					
Phase I (3 yr. averages)	48 000	34 000	70 000	152 000	44 000
% of costs covered by revenue	0	0	63%	28%	
(b)					
Phase II (average)	77 000	52 000	123 000	252 000	153 000
% covered by revenue	40%	0	100%	60%	
(c)					
Year 6 1991/92	80 000	40 000	127 000	247 000	191 000
% covered by revenue	81%	0	100%	77%	

93. Thus, the financial projections indicate that in the sixth year 77 per cent of all project costs, including depreciation, will be covered by revenue. There will be a recurrent deficit of P 56 000 on total expenditure of P 247 000, with all commercial and 81% of administrative costs covered by project revenues. The greater part of the P 56 000 deficit is attributable to the costs of demonstration and extension. These costs are amply justified by the fact that the nett cash incomes of outgrowers in 1991/92 are projected in Table 10 to exceed P 200 000 in 1985 pula.

94. Some explanation may be required of the annual allowance of 15 per cent which has been added to all estimates to cover both contingencies and inflation. This provision may well prove to be inadequate where estimated costs are concerned.The collapse of the South African rand brought about a 75 per cent decline in the foreign exchange value of the Botswana pula against all major currencies except the rand in 1985. This decline may well continue in 1986. Pula prices of all imported goods began to rise steeply in Botswana at the end of 1985. The inflation rate in South Africa (the source of 90 per cent of Botswana's imports) is forcast to rise to an annual rate of 25% in 1986.

95. As these inflationary and foreign exchange effects work through the Botswana economy in 1986 there will be upward pressure on wages, salaries and the prices of all main agricultural inputs. To the extent, however, that Thusano Lefatsheng succeeds in developing overseas markets for its herbal, aromatic and medicinal products, there will be a compensating foreign currency gain, i.e. the pula price of exports to overseas countries will be greatly increased. Thusano Lefatsheng's projected high-value low-bulk exports (particularly to the EEC where they can enter duty free under Lomé II) now promise to be highly competitive in relation to suppliers of similar products from all other known producing countries.

TABLE 1

Capital Items Phase I. 1986 - 1989

Item	Year 86/87	87/88	88/89	Total
Staff Houses 2 of 65m² ea.	--	10,000	10,000	20,000
Guest Houses 2 Of 30m² ea.	10,000	--	--	10,000
Employees'houses 10 of 25m² ea.	7,500	4,500	4,500	16,500
Offices and Laboratory 50m²	5,000	5,000	--	10,000
Workers'Kitchen + rest area	2,000	--	--	2,000
Inputs Store 40m²	4,000	1,000	--	5,000
Workshops 60m²	6,000	2,000	2,000	10,000
Tool Store 10m²	--	1,000	--	1,000
Nursery School	--	--	4,000	4,000
Drying + Storage sheds and racks 70m²	10,000	2,000	2,000	14,000
Net houses 2x250m²	2,000	2,000	--	4,000
Processing and packing equip.	3,000	2,000	2,000	7,000
Laboratory Equipment	2,500	2,500	5,000	10,000
Cultivation Machinery + tools	9,000	9,000	9,000	27,000
Workshop tools	9,000	5,000	4,000	18,000
Water Reservoirs 100m³	6,000	6,000	--	12,000
Water reticulation	6,000	6,000	12,000	24,000
Water Treatment	2,000	2,500	--	4,500
Electric power supply	6,000	--	18,000	24,000
Pickups (2)	14,000	16,000	--	30,000
Motor cycles (2)	--	2,500	2,500	5,000
Bicycles (4)	500	--	--	500
Office Equipment	500	1,000	1,000	2,500
Sub totals	105,000	80,000	76,000	261,000
Contingencies and inflation 15%	15,750	12,000	11,400	39,150
SUB-TOTAL	120,750	92,000	87,400	300,150
Funds for initial purchases of plant materials from gatherers and outgrowers (working capital)	10,000	10,000	--	20,000
Revolving Loan Fund (working capital)	--	25,000	25,000	50,000
TOTAL CAPITAL ASSISTANCE REQUESTED, STAGE I	130,750	127,000	112,400	370,150

TABLE 2

NOTIONAL BREAKDOWN OF PHASE I CAPITAL REQUEST BY BUDGET CATEGORY, viz (a) Project Administration; (b) Extension, Research and Evaluation; (c) Commercial Operations.

Item	(a) Admin	(b) Ext. Res.,etc	(c) Comm.	(d) Total Phase I Capital
	P	P	P	P
Housing	25,000	3,000	18,500	46,500
Offices + Laboratory	8,000	2,000	--	10,000
Kitchen,etc	2,000	--	--	2,000
Inputs Store	--	--	5,000	5,000
Workshops	4,000	--	6,000	10,000
Tool Store	--	--	1,000	1,000
Nursery School	4,000	--	--	4,000
Drying,Storage and shed	--	--	14,000	14,000
Nethouse (2)	--	2,000	2,000	4,000
Processing + pack.Equip.	--	--	7,000	7,000
Laboratory Equipment	--	10,000	--	10,000
Machinery + Tools	--	7,000	20,000	27,000
Workshop Tools	--	--	18,000	18,000
Water Res.	2,000	2,000	8,000	12,000
Water Ret.	3,000	3,000	18,000	24,000
Water Treat.	4,500	--	--	4,500
Electric pow	2,000	2,000	20,000	24,000
Vehicles (2)	10,000	10,000	10,000	30,000
Motor Cycles (2)	2,500	2,500	--	5,000
Bicycles(4)	125	250	125	500
Office Equip.	2,500	--	--	2,500
Sub-Totals	69,625	43,750	147,625	261,000
15% Conting. & inflation	10,450	6,550	22,150	39,150
Work.Capit.	--	5,000	15,000	20,000
Rev.Loan Fund	--	25,000	25,000	50,000
TOTALS	80,075	80,300	209,775	370,150
(% of Total)	(22%)	(22%)	(56%)	(100%)

TABLE 3

Capital Items, Phase II, 1989-1992

Item	Year 89/90	90/91	91/92	Total
Staff Houses 2 of 65m² ea.	12,000	12,000	--	24,000
Drying Shed + Store rooms extension 70m²	10,000	--	--	10,000
Processing + Packing equip.	10,000	10,000	--	20,000
Laboratory equipment	2,000	2,000	--	4,000
Cultivation tools + equipment	8,500	8,500	--	17,000
Workshop tools	1,500	1,500	1,500	4,500
Water Reservoir extension	4,000	4,000	4,000	12,000
Water Reticulation extensions	5,000	5,000	5,000	15,000
8 seat combi (extension)	18,000	--	--	18,000
Office Equipment	500	500	--	1,000
Sub-total	71,500	43,500	10,500	125,500
Contingencies and inflation 15%	10,725	6,525	1,575	18,825
TOTAL CAPITAL ASSISTANCE REQUESTED, STAGE II	82,225	50,025	12,075	144,325

TABLE 4

NOTIONAL BREAKDOWN OF PHASE II CAPITAL REQUEST BY BUDGET CATEGORY

Item	(a) Admin. P	(b) Ext. Res.,etc. P	(c) Comm. P	(d) Total Phase II Capital P
Housing	8,000	8,000	8,000	24,000
Drying Sheds + Store rooms	--	2,000	8,000	10,000
Processing + Pack.Equip.	--	--	20,000	20,000
Laboratory Equipment	--	4,000	--	4,000
Tools & Equip.	--	--	21.500	21,500
Water Reserv.	--	4,000	8,000	12,000
Water Reticu.	--	5,000	10,000	15,000
8 Seat Combi	--	18,000	--	18,000
Office Equip.	1,000	--	--	1,000
Sub Totals	9,000	41,000	75,500	125,500
Cont.% Infl. 15%	1,350	6,150	11,325	18,825
TOTALS	10,350	47,150	86,825	144,325
(% of Total)	(7%)	(32%)	(61%)	(100%)

TABLE 5

Recurrent Cost Estimates Phase I 1986 - 1989

Item	Year			
	86/87	87/88	88/89	Total
Seeds, plants, containers	2,000	2,000	2,000	6,000
Packing Materials	2,000	2,000	3,000	7,000
Books + stationery	1,000	1,000	1,000	3,000
Manures, sprays, etc.	3,500	2,500	2,500	8,500
Market Research	2,000	2,000	3,000	7,000
Vehicle + equip. operating costs	10,000	10,000	20,000	40,000
Administrative overheads	3,000	3,000	4,000	10,000
Accountant's fees	1,000	1,000	1,000	3,000
Audit fees	1,000	2,000	2,000	5,000
Wages 10 labourers	12,000	12,000	12,000	36,000
Casual Labour	12,000	14,000	20,000	46,000
1 foreman	1,600	1,600	1,600	4,800
1 Cook	1,000	1,000	1,000	3,000
Salaries: Manager	18,000	18,000	18,000	54,000
Agricultural Manager*	8,000	8,000	8,000	24,000
Assistant Manager(Administ.)	12,500	12,500	--	25,000
Counterpart Assistant Manag:**	9,000	9,000	12,500	30,500
Assis,Manager (Marketing)	--	--	12,000	12,000
Secretary/Bookkeeper	6,000	6,000	6,000	18,000
Analysis of Plants ***	2,000	2,000	--	4,000
Ecological and Bot. surveys	10,000	10,000	2,000	22,000
Crop Research	2,500	2,500	5,000	10,000
Sinking fund for vehicle/ Machinery replacement (Depreciation)	--	10,000	10,000	20,000
Sud Totals	120,100	132,100	146,600	398,800
Conting. and Inflation 15%	18,000	19,800	22,000	59,800
TOTAL	138,100	151,900	168,600	458,600
Less Est.Project Income (See Table)	14,000	45,600	72,300	131,900
NET LOSS (= TOTAL RECURRENT ASSISTANCE REQUESTED, STAGE I)	124,100	106,300	96,300	326,700

Note to Recurrent Costs, Phase I :

* The Agricultural Manager will have a salary of P 18,000 a year, of which P 10,000 will be paid by U.S.AID for three years and P 8,000 by this project.

** The Counterpart Assistant Manager will replace the Assistant Manager (expatriate) in 1987/89.

*** Analysis of plants will be performed free of charge by Universit of Botswana from 1987/1988 onwards.

TABLE 6

NOTIONAL BREAKDOWN OF PHASE I RECURRENT ESTIMATES BY BUDGET CATEGORY

Item	(a)Admin.	(b) Ext.Res. etc.	(c)Comm.	(d) Total Phase I Recurrent
	P	P	P	P
Seeds,plants, etc.	--	2,000	4,000	6,000
Pack. Materials	--	--	7,000	7,000
Books,stationery	2,000	1,000	--	3,000
Manures,sprays	--	1,000	7,500	8,500
Market Research	--	7,000	--	7,000
Vehic.&Equip. operating costs	14,000	6,000	20,000	40,000
Admin.Overheads	10,000	--	--	10,000
Accountant's Fees	3,000	--	--	3,000
Audit Fees	5,000	--	--	5,000
Wages:				
10 labourers	3,600	5,000	27,400	36,000
Casual labour	2,000	3,000	41,000	46,000
Foreman	--	--	4,800	4,800
Cook	3,000	--	--	3,000
Salaries:				
Manager	20,000	14,000	20,000	54,000
Agr,Manager	--	6,000	18,000	24,000
Asst.Manager	10,000	5,000	10,000	25,000
Count,Asst.Man.	15,000	3,500	12,000	30,500
Marketing Man.	--	--	12,000	12,000
Secretary/& Bookkeeper	18,000	--	--	18,000
Analysis of Plants	--	4,000	--	4,000
Ecol. & Bot. Surveys	--	22,000	--	22,000
Crop research	--	10,000	--	10,000
Sinking Fund (Depreciation)	20,000	--	--	20,000
Sub Totals	125,600	89,500	183,700	398,800
Contingencies &inflat.@15%	18,825	13,425	27,550	59,800
TOTALS	144,425	102,925	211,250	458,600
(% of Total)	(31%)	(22%)	(47%)	(100%)

TABLE 7

Recurrent Cost Estimates Phase II, 1989 - 1992

Item	Year 89/90	90/91	91/92	Total
Seeds,Plants,Containers	2,000	2,000	2,000	6,000
Packing Materials	3,000	3,000	3,000	9,000
Books + stationery	1,000	1,000	1,000	3,000
Manure,sprays,etc.	2,500	2,500	2,500	7,500
Market Research	4,000	4,000	4,000	12,000
Vehicle + equipment operating	26,000	28,000	30,000	84,000
Administrative overheads	5,000	5,000	5,000	15,000
Accountant's fees	2,000	2,000	2,000	6,000
Audit fees	3,000	3,000	3,000	9,000
Wages : 10 labourers	12,000	12,000	12,000	36,000
Casual Labour	26,000	26,000	26,000	78,000
1 Foreman	2,000	2,000	2,000	6,000
1 Cook	1,000	1,000	1,000	3,000
1 Creche Supervisor	1,200	1,200	1,200	3,600
Salaries:				
Manager	18,000	18,000	18,000	54,000
Agricultural Manager	18,000	18,000	18,000	54,000
Assist.Manager (Agric.)	12,000	12,000	12,000	36,000
Assist.Manager (Adminis.)	12,500	12,500	12,500	37,500
Assist.Manager (Mechanical)	12,000	12,000	12,000	36,000
Assistant Manger(Marketing)	12,000	12,000	12,000	36,000
Secretary/Bookkeeper	6,000	6,000	6,000	18,000
Evaluation of Phase I	20,000	--	--	20,000
Ecol. & Botanical Surveys	4,500	4,500	4,500	13,500
Crop Research	5,000	5,000	5,000	15,000
Sinking Fund (Depreciation)	20,000	20,000	20,000	60,000
Sub-Totals	230,700	212,700	214,700	658,100
Conting. & Inflation @ 15%	34,600	31,900	32,200	98,700
Total	265,300	244,600	246,900	756,800
Less:Est. Project Income (see Table)	108,600	148,000	202,000	458,600
NET LOSS (=TOTAL RECURRENT ASSISTANCE REQUESTED, STAGE II)	156,700	96,600	44,900	298,200

TABLE 8

NOTIONAL BREAKDOWN OF PHASE II RECURRENT ESTIMATES BY BUDGET CATEGORY

Item	(a) Admin.	(b) Extens. Res. etc.	(c) Comm.	Total Phase I Recurrent
	P	P	P	P
Seeds,Plants,etc.	---	1,000	5,000	6,000
Packing Materials	---	---	9,000	9,000
Books & Stationery	2,250	750	---	3,000
Manure,Sprays	---	1,000	6,500	7,500
Market Research	---	12,000	---	12,000
Vehic.&Equip.oper.	28,000	14,000	42,000	84,000
Adminis.Overheads	15,000	---	---	15,000
Accountant's Fees	6,000	---	---	6,000
Audit Fees	9,000	---	---	9,000
Wages:				
10 labourers	3,600	2,400	30,000	36,000
Casual labour	4,000	4,000	70,000	78,000
Foreman	1,000	1,000	4,000	6,000
Cook	3,000	---	---	3,000
Creche sup.	3,600	---	---	3,600
Salaries:				
Manager	20,000	14,000	20,000	54,000
Agric.Manager	---	12,000	42,000	54,000
Asst.Man.(Agric)	---	6,000	30,000	36,000
Asst.Man,(Admin)	20,000	7,500	10,000	37,500
Asst.Man.(Mech)	8,000	---	28,000	36,000
Asst.Man.(Mark)	---	12,000	24,000	36,000
Secre./Bookkeep.	18,000	---	---	18,000
Phase I Evaluat.	---	20,000	---	20,000
Ecol.&Bot.Surveys	---	13,500	---	13,500
Crop Research	---	15,000	---	15,000
Depreciation	60,000	---	---	60,000
Sub-Totals	201,450	136,150	320,500	658,100
Cont.+ Infl.15%	30,200	20,400	48,100	98,700
TOTALS	231,650	156,550	368,600	756,800
(% of Total)	(32%)	(20%)	(48%)	(100%)

TABLE 9

Estimated Gross Income to Thusano Lefatsheng from own production (in December 1985 pula) 1986-1993

Year	10 Ha low ground production unit	50 Ha Hillside unit	Marketing of Gathered Wild Products	Marketing of Outgrower Produce	Total
86/87	10 000	--	4 000	--	14 000
87/88	30 000	--	6 000	9 600	45 600
88/89	40 000	5 000	8 000	19 300	72 300
89/90	50 000	10 000	10 000	38 600	108 600
90/91	60 000	20 000	10 000	58 000	148 000
91/92	70 000	25 000	10 000	87 000	192 000
92/93	80 000	30 000	15 000	116 000	271 000
TOTALS	340 000	90 000	63 000	328 500	821 500

TABLE 10

Estimated investment costs and net incomes to Outgrowers and Gross income to Thusano Lefatsheng from Marketing of Outgrowers production (in 1984 pula), 1986-1993

Year	Cumulative No. of Outgrowers	Annual New Invest. by Outg*	Annual No of Paid Jobs Created in outg. Hseholds	Annual Gross Sales	Net Returns to Outgro, (after Wages)	Annual Gross Income to T.L at 20% of Sales (84Pula)	(Dec 85 Pula + 33%)
86/87	--	--	--	--	--	--	--
87/88	5	43 000	15	36 275	8 600	7 250	9 6C
88/89	10	43 500	15	72 550	17 390	14 500	19 3C
89/90	20	87 000	30	145 100	34 780	29 000	38 0C
90/91	30	87 000	30	217 650	52 170	43 500	58 00(
91/92	45	130 500	45	326 475	78 255	65 300	87 00(
92/93	60	130 500	45	435 300	104 340	87 100	116 00
TOTALS	60	522 000	180	1 233 345	295 535	246 650	327 90

* Investment from own resources, government grant % subsidy schemes, and loans.

Source: Product profiles (see Appendix II) for smallholder growing of chillie pepers, leaf and snuff tobacco, and morogo.

1592 Deutsche Apotheker Zeitung 120. Jahrg. Nr 34 21. 8. 1980

ANNEX 7

Quantitative Bestimmung von Harpagosid in Wurzeln von Harpagophytum procumbens mit Hochleistungsflüssigkeits-chromatographie (HPLC)

Von Otto Sticher und Beat Meier*, Zürich

Einige Iridoid- und Secoiridoidglucoside enthaltende Pflanzen behaupten seit langem ihre Stellung als Arzneimittel. Dazu gehören Plantago lanceolata, Harpagophytum procumbens, Valeriana officinalis, Gentiana-Arten u. a. Sie haben eine entsprechende pharmazeutische Bedeutung und sind als Arzneipflanzen heute dem Arzneimittelgesetz unterstellt. Dies bedingt, daß sie eine gesicherte Qualität aufweisen (2). Die Gehaltsbestimmung der wichtigsten Inhaltsstoffe von Arzneipflanzen erhält entsprechend zusehends größere Bedeutung.

Harpagosid gehört zur Gruppe der iridoiden Pflanzenstoffe, im speziellen zu den Iridoidglucosiden. Diese sind in neuester Zeit wegen ihrer chemotaxonomischen, pharmazeutischen und biochemischen Bedeutung immer aktueller geworden. Übersichtsarbeiten jüngsten Datums erschienen von Hegnauer und Kooiman (3) über die chemotaxonomischen Aspekte, von Inouye (4) über den neuesten Stand der Biosynthese, von Rimpler (5) über die Strukturaufklärung und von Sticher (6) über die pharmakologische, biologische und pharmazeutische Aktivität.

Sticher (7) beschäftigte sich in einer Arbeit über die Gehaltsbestimmung von Iridoiddrogen auch mit dem Nachweis von Harpagosid in Harpagophytum procumbens DC. Ebenso gab er eine Übersicht über den Stand der Kenntnisse der Droge Tubera (Radix) Harpagophyti (8). Darin (7,8) sind auch die ersten Resultate mit einer neuen HPLC-Methode zur quantitativen Bestimmung des Harpagosids aufgeführt, von der erstmals 1976 in München berichtet wurde (9). Da Harpagosid an der antiphlogistischen und analgetischen Wirkung der Droge mitbeteiligt ist ([8] und darin zit. Lit., [10]), ist es verständlich, daß in der Zwischenzeit auch weitere Methoden zur Bestimmung von Harpagosid entwickelt worden sind.

Haag-Berrurier et al. (11) beschrieben eine kolorimetische Bestimmung des Iridoidgesamtgehaltes nach Reaktion mit Vanillin-Schwefelsäure. Czygan et al. (12) bestimmten das Harpagosid nach dünnschichtchromatographischer Abtrennung semiquantitativ bzw. nach Elution des chromatographisch getrennten Harpagosids spektrophotometrisch mit Hilfe einer Eichkurve. Erdös et al. (10) verwendeten die HPLC im Rahmen von pharmakologischen Untersuchungen zur Bestimmung des Harpagosid-Gehaltes der von ihnen getesteten Präparate.

Die kolorimetrische Methode nach Haag-Berrurier ist zwar einfach durchzuführen und benötigt keine besonderen Apparaturen, weist aber eine Reihe der von uns (7,8) früher beschriebenen Nachteile auf. Auch andere Methoden auf der Basis der bekannten Aucubin-Bestimmungen erbrachten in bezug auf die Reproduzierbarkeit keine zufriedenstellenden Resultate (1). Erst die direkte spektrophotometrische Bestimmung des Harpagosids nach dünnschichtchromatographischer Trennung auf Kieselgelplatten kennt die Nachteile der Farbreaktionsmethoden nicht. Die Eichgerade verläuft linear durch den Nullpunkt. Die Methode ist empfindlich, 25 bis 100 µg Harpagosid in 10 ml Meßlösung genügen zur Analyse.

*Teil der Dissertation von B. Meier, ETH Zürich, 1978 (1)

Die statistischen Daten beweisen die besseren Eigenschaften dieser Eichgeraden gegenüber jenen der Farbreaktionen (1).

Gehaltsbestimmung mit der HPLC

Mit der Hochleistungs- oder Hochdruckflüssigkeitschromatographie (HPLC) bietet sich eine analytische Methode an, welche sich durch ihre Einfachheit in der Durchführung, Schnelligkeit und Genauigkeit auszeichnet. Als Trennsystem für die gut wasserlöslichen Iridoidglucoside eignet sich am besten eine Reversed Phase-Säule mit einem polaren Elutionsmittel. Es wird direkt ein Extrakt in die Säule eingespritzt. Nach etwa 5 Minuten kann der Harpagosid-Gehalt berechnet oder an einem Integrator direkt abgelesen werden. Die Detektion des Harpagosids mit einem UV-Spektrophotometer bietet keine Schwierigkeiten, da das Absorptionsmaximum von 278 nm höher ist als dasjenige der mit Methanol extrahierten Begleitstoffe. So konnte Harpagosid im Chromatogramm des Extraktes als deutlicher Hauptpeak detektiert werden. Eine selektive Extraktionsmethode ist entsprechend nicht notwendig. Je nach Drogenmuster sieht das Chromatogramm im unteren k'-Bereich unterschiedlich aus (Abb. 1).

Um Zersetzungen möglichst zu vermeiden, wurde zuerst kalt, dann heiß extrahiert. Nach zweimaliger Extraktion mit je 50 ml Methanol konnte in einem dritten Nachextrakt mit HPLC praktisch kein Harpagosid (<2 % des Gesamtgehaltes) mehr nachgewiesen werden.

Die mobile Phase mußte, bedingt durch die aromatische Struktur des Harpagosids, relativ viel Methanol enthalten. Mit 50 % Methanol in Wasser konnte der Harpagosid-Peak in genügendem Ausmaß von den übrigen Stoffen im Extrakt abgetrennt werden. Die Nachweisgrenze von Harpagosid liegt bei 0,2 µg (injiziert in 10 µl), Absorbance 0,1 A.

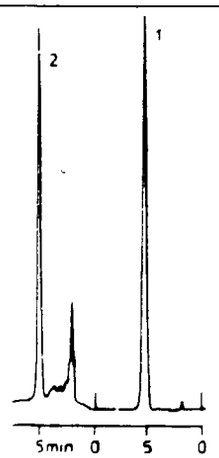


Abb. 1: HPLC-Chromatogramm von Tubera Harpagophyti
Mobile Phase: Methanol-Wasser (50:50)
Durchflußgeschwindigkeit: 2 ml/min
1 = Harpagosid-Referenz, 2 = Harpagosid-Peak in einem Extrakt von Tubera Harpagophyti

Extraktion der Droge

500 mg pulverisierte, getrocknete Droge wurden mit 50 ml Methanol während einer Stunde unter Rühren kalt extrahiert. Die Lösung wurde durch ein G4-Filter filtriert. Das Pflanzenmaterial wurde ein zweites Mal während einer Stunde, diesmal bei 70 °C am Rückflußkühler, mit 50 ml Methanol extrahiert, danach filtriert und ausgewaschen. Die Methanolfraktionen wurden in einem Rundkolben vereinigt und auf 2 bis 3 ml eingeengt (Temperatur maximal 40 °C). Dabei fielen etliche Ballaststoffe aus. Der verbliebene Extrakt wurde filtriert und in einen 10,00 bzw. 20,00 ml-Meßkolben übergeführt. Mit Methanol wurde bis zur Marke aufgefüllt. 10 bzw. 20 µl der Analysenlösung wurden ins HPLC-System injiziert.

Chromatographische Bedingungen und verwendete Apparaturen

A) Dünnschichtchromatographie (13):
Platte: Kieselgel 60 F 254 Fertigplatten der Firma Merck
Fließmittel: Chloroform p. a.–Äthanol 94 % (2 1). Kammersättigung
Rf-Wert Harpagosid: 0,55
Detektion: UV-Licht 254 nm (Fluoreszenzminderung) und Phloroglucin-Salzsäure Reagens (standardisiert nach [14])
Dosiergerät für quantitative Dünnschichtchromatographie: Desaga Microdoser® nach Dibbern

B) Hochdruckflüssigkeitschromatographie
Verwendet wurden: Pumpe, Waters Model M-6000A (Waters Assoc., Milford, Mass., USA); Injektor, Waters, Model U6K; Säule, Waters µBondapak C_{18} (P/N 27324) 3,9 mm I. D. x 30 cm; Detektoren, Beckman Model 25 (Beckman, Fullerton, CA, USA) mit variabler Wellenlänge und Waters-LC-25-Mikrozelle, sowie Perkin-Elmer LC 55 (Coleman, Maywood, Ill., USA). Calculator, Hewlett-Packard Model 9830A; Printer, Hewlett-Packard Model 9866A und Digitizer, Hewlett-Packard Model 9864A (Hewlett-Packard Calculator Product Division, Colorado, USA).

Als mobile Phase wurde Methanol–Wasser (50:50) verwendet. Die Analysen wurden bei Raumtemperatur bei einer Durchflußgeschwindigkeit von 2 ml/min und einem Druck von etwa 4000 psi durchgeführt. Die Detektion erfolgte bei 278 nm (Absorbance 0,5 A). Die Retentionszeit für Harpagosid betrug 5 bis 6 Minuten, die gesamte Analysenzeit etwa 10 Minuten. Jeweils nach 6 bis 8 Analysen wurde die Säule mit Methanol durchgespült. Die theoretische Bodenzahl für Säule 1 betrug 700 und für Säule 2 = 1250 (bezogen auf Harpagosid).

Die Reinheit des Harpagosid-Peaks sowie die Identität mit der Reinsubstanz wurde wie folgt kontrolliert: Dreimal 50 µl Extrakt wurden auf der HPLC-Säule chromatographiert und das abgetrennte Harpagosid am Ende der Säule aufgefangen. Nach Abdampfen des Lösungsmittels wurde der Rückstand in 150 µl Methanol gelöst und dünnschichtchromatographisch mit Referenzsubstanz verglichen. Ebenfalls wurde die Identität des Harpagosid-Peaks mit der Stop-flow-Methode (UV identisch mit Harpagosid-Referenz, λ_{max} = 278 nm) bewiesen.

Abb. 2: HPLC-Chromatogramm von Tubera Harpagophyti zur quantitativen Bestimmung (Ausmessung der Fläche)
Mobile Phase: Methanol–Wasser (50:50)
Durchflußgeschwindigkeit: 2 ml/min
Papiervorschub bis ¸ 5 mm/min, ab ¸ 3 cm/min

Auswertungsverfahren

Gearbeitet wurde mit der Methode des externen Standards, was genaue Flußkontrolle und präzise Injektionen erforderte. Zur Messung der Peakflächen wurde jeweils der Papiervorschub des Schreibers erhöht (Abb. 2): von 0,2 auf 1 inch/min (Beckman, Model 25) respektive von 0,5 auf 3 cm/min (Perkin-Elmer LC 55). Die Peakflächenmessung wurde mit einem Digitizer vorgenommen. Die Peakfläche wurde anstelle der Höhe gewählt, weil die Peaksymmetrie der ersten Säule nicht optimal war (1).

Für die Auswertung wurde wie folgt verfahren:
A) Man erstellte eine Eichgerade durch wiederholtes Einspritzen verschiedener Mengen von Harpagosid. Mit der Gleichung der Eichgeraden (korrigierte Regression) und der aus mindestens zwei Analyseneinspritzungen gleichen Volumens ermittelten mittleren Peakfläche wurde der Gehalt bestimmt. Die Peakhöhe sollte dabei mindestens 50 % des Vollausschlages überschreiten. Die Eichgerade bleibt über längere Zeit konstant. Dies muß regelmäßig kontrolliert werden.
B) Chromatographiert wurden abwechslungsweise Extrakt und genau definierte Mengen von Referenzsubstanz. Der Referenzpeak sollte etwa die gleiche Größe aufweisen wie der Extraktpeak. Die Formel zur Berechnung lautet:

$$\text{Gehalt in mg} = \frac{F_H}{F_{St}} \cdot E_{St} \cdot \frac{I}{V} \cdot 10^3$$

$$\text{Gehalt in \%} = \frac{F_H}{F_{St}} \cdot E_{St} \cdot \frac{I}{V} \cdot \frac{10^5}{E_H}$$

F_{St} = Peakfläche Standardsubstanz (Harpagosid)
F_H = Peakfläche Harpagosid aus Extrakt
E_{St} = Eingespritzte Menge Standardsubstanz (µg)
E_H = Einwaage Droge (in mg)
I = µl eingespritzter Drogenextrakt
V = Volumen Drogenextrakt (ml)

Eichgeraden

Verschiedene Stammlösungen von 15 bis 20 mg Harpagosid in 25,00 ml Methanol wurden hergestellt. 8 bis 12 µl davon wurden injiziert und die erhaltenen Peakflächen ausgemessen. Die Haltbarkeit der Stammlösungen im Kühlschrank betrug einen bis zwei Monate.

Mit einer Regressionsanalyse wurde der lineare Zusammenhang zwischen Einspritzmenge an Harpagosid (x = µg Harpagosid) und Peakfläche (y = Fläche in mm^2) bewiesen (1).

Ergebnisse der Gehaltsbestimmung

Es wurden jeweils frisch hergestellte Extrakte analysiert. Die Auswertung erfolgte mit der Eichgeraden y = 270,7 x. Die Ergebnisse der Gehaltsbestimmung sind in Tabelle 1 zusammengestellt.

Am Beispiel des Drogenmusters 1 wurde untersucht, ob die Auswertung mit der Eichgeraden y = 270,7 x andere Resultate ergibt als die Auswertung mit dem Vergleich der Peakflächen. Dabei konnte nachgewiesen werden, daß die beiden Methoden keinen signifikanten Unterschied ergeben (15,7 ± 1,0 bzw. 15,9 ± 1,0 mg Harpagosid pro g Droge; Mittelwert von sechs Extrakten zu je drei Bestimmungen).

Am Beispiel des Reinstoffes Harpagosid wurde untersucht, ob die Auswertung mit Hilfe der Peakhöhe andere Resultate ergibt als mit der Peakfläche. Die Ergebnisse zeigten, daß sowohl die Höhenmeß-

Tab. 1: Ergebnisse der Gehaltsbestimmung

Drogenmuster[1]	Extrakte/ Bestimmungen[2]	Gehalt mg/g Droge	%
1	9/3	15.8 ± 0.8	1.58 ± 0.08
2	7/3	14.9 ± 0.7	1.49 ± 0.07
3	2/3	16.3 ± 0.8	1.63 ± 0.08
4	2/3	15.3 ± 0.7	1.53 ± 0.07
5	2/3	15.4 ± 0.7	1.54 ± 0.07
6	2/3	15.2 ± 0.7	1.52 ± 0.07

[1] Die Drogenmuster wurden im Mai 1976 aus verschiedenen Apotheken der Schweiz bezogen.
Makroskopisch unterschied sich nur Muster 2 von den übrigen. Die Analysen wurden zwischen Mai und August 1976 durchgeführt.

[2] Anzahl Extrakte (erste Zahl) die je x mal (zweite Zahl) bestimmt wurden.

methode wie die Flächenmeßmethode eingesetzt werden können. Bei Peaks mit geringen Höhen wird allerdings die Genauigkeit bei Verwendung der Flächenmeßmethode besser.

Am Beispiel der Drogenmuster 1, 5 und 6 wurde untersucht, ob ein Einfluß auf den Gehalt ermittelt werden kann, falls mit verschiedenen UV-Detektoren (Beckman Model 25 bzw. Perkin Elmer LC 55) gearbeitet wird. Dabei ergab sich, daß die Resultate, selbst wenn verschieden alte Säulen verwendet wurden, innerhalb der Fehlergrenzen von ± 5 % für die gesamte Analyse reproduzierbar sind (Muster 1 = 15.9 ± 0.4 bzw. 16.9 ± 0.3, Muster 5 = 16.1 ± 0.3 bzw. 16.6 ± 0.3; Muster 6 = 14.7 ± 0.2 bzw. 15.7 ± 0.3. Gehalt in mg Harpagosid pro g Droge).

Diskussion der Methode und der Ergebnisse

Auch mit einer nicht optimalen Säule sind die Ergebnisse der Methode reproduzierbar. Innerhalb der gleichen Probe liegen die Standardabweichungen (relativ) unterhalb von 2 %. Vergleicht man Analysen verschiedener aus einem Drogenmuster hergestellter Extrakte, werden die Fehler wesentlich größer, summieren sich doch die einzelnen Fehlermöglichkeiten (Einwaage, Verdünnung, Injektion, Unregelmäßigkeiten beim Durchfluß der mobilen Phase). Der Harpagosidgehalt kann so mit einer relativen Standardabweichung von ± 5 % ermittelt werden. Reduzieren ließe sich der Fehler durch die Anwendung vollautomatischer Injektions- und Auswertungssysteme und durch Anwendung der Methode des inneren Standards.

Abgesehen vom apparativen Aufwand kann die HPLC-Methode jener der Dünnschichtchromatographie Spektrophotometrie, sofern dabei die Auswertung nicht automatisch erfolgen kann, als gleichwertig, vom Zeitbedarf her als überlegen gegenübergestellt werden. Bei Verwendung neuer Säulen ist die Retentionszeit des Harpagosids um einige Minuten größer. Die zur Analyse verwendeten Säulen zeigten im Betrieb einen später bei neueren Säulen nicht mehr konstatierten Effekt. Mit der Zeit stabilisierten sich die Kapazitätsverhältnisse k' für Harpagosid auf einem Wert, der deutlich unter jenem bei Inbetriebnahme der Säule lag. Die Auflösung wurde dadurch nicht beeinflußt.

Zum Nachweis der übrigen Indoidglucoside in Tubera Harpagophyti (Harpagid, Procumbid) muß eine polarere mobile Phase mit weniger Methanol eingesetzt werden.

Wir danken Herrn Daniel Lehmann, Pharmazeutisches Institut, ETH Zürich, für die Isolierung von reinem Harpagosid. Dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung danken wir für die teilweise Unterstützung der vorliegenden Arbeit.

Summary

Quantitative determination of harpagoside in the roots of Harpagophytum procumbens with High Performance Liquid Chromatography (HPLC). A HPLC-method for quantitative determination of harpagoside in Harpagophytum procumbens was developed. A reversed-phase-system with μBondapak C_{18} column using methanol–water (50:50) as eluent was used. The method is adaptable for routine analysis of Harpagophytum extracts. The detection limit for harpagoside was at 278 nm about 0.2 μg/10 μl. The relative standard deviation was between 2 and 5 %.

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ANNEX 8

Plants to be cultivated or collected

1. Species with established market

Harpagophytum procumbens - no upper limit.

2. Species for use in the primary health care in Botswana

Euphorbia hirta - expectorant — for a cough
Glaucium flavum - antitussive — syrup
Securinega longipeduculata - expectorant

Argemone mexicana - sedative

Urginea altissima — cardiac glycosides
Asclepias fruticosa — (substitute for Digitalis)
(Gomphocarpus)

Chenopodium ambrosioides - essential oil: antihelmintic

Datura innoxia - scopolamine

Eucalyptus globulus - essential oil as antibronchitis

Hibiscus subdariffa - for tea (color + taste)

3. Potential export in ton-quantities

Echinacia purpurea - herba
Centella asiatica - herba
Passiflora incarnata - herba
Symphytum officinalis - radix
Rauwolfia vomitoria - radix + folium
Tribulus terrestis - herba
Bixa orellana - semen

4. Potential export of essetial oils from:

Eucalyptus globulus
Mentha piperita
Rosmarinus officinalis
Salvia officinalis
Thymus vulgaris
Foeniculum vulgaris
Lavendula officinalis

5. Potential use in Botswana and export in minor quantites

Capsicum frutescens
Aloe vera
Artemisia afra
Calendula officinalis
Cassia acutifolia
Chrysanthemum parthenium
Cynara scolymus
Gypsophila paniculata
Saponaria officinalis
Oreganum majoranum
Rosmarinus officinalis
Thymus vulgaris
Valeriana officinalis
Verbeana citratum
Lantana camara