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ASSISTANCE FOR THE PRODUCTION OF PLANT-DERIVED PHARMACEUTICALS

DP/URT/81/026

UNITED REPUBLIC OF TANZANIA

Technical report: Analyses of Therapeutic Agents in Traditional Medicine *

Prepared for the Government of Tanzania
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Programme

Based on the work of Alfred T.Elvin,
Expert in instrumental analysis and quality control

United Nations Industrial Development Organization
Vienna

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I. SUMMARY

This report concerns installation and maintenance of analytical instrumentation in the Traditional Medicine Research Centre. It also addresses equipment and quality control procedures needed to meet the requirements of a modern research laboratory. A working plan is presented for screening and purity assessment of plant derived medicinals. Finally, a plan for future training of laboratory personnel is presented.

II. RECOMMENDATIONS

1. To The Traditional Medicine Research Centre

   a. Equipment

      The Laboratory now possesses or has ordered most of the capital equipment needed to implement screening and quality control. However, some additional Equipment is needed and is listed in Appendix 2.

   b. Disposable Supplies

      Supplies for one to two years operation have been ordered with the exception of some additional reagents, gases, and HPLC solvents. Operation of the Laboratory will require a continuous and reliable source of these commodities and the funds necessary for their purchase.

c. Quality Control Standards

Sources of standard samples of investigational drugs and essential oils must be identified (ie, USP-UNITED STATES, ROMANIA, INDIA). These standards should have accompanying GLC or HPLC profiles with major peaks identified. Rigid storage and purity check procedures for these standards are absolutely necessary.

d. Laboratory

A dehumidifier should be installed in the instrument room. Also, windows should be sealed with plastic to exclude outside dust. Cabinets should be obtained for storage of spare parts. A four-drawer file cabinet is needed for storage of chromatographic data.

e. Energy Supply

Further efforts should be made to provide a stable and continuous power supply to instruments and sample storage refrigerators. Since power interruption occurs almost daily, a portable generator capable of running refrigeration and instrumentation equipment should be considered.

f. Outside Ties

Close cooperation with one or two universities in Europe and one university in the United States is recommended for exchange of ideas and research results, access to latest literature, literature searches, and postgraduate education.

g. Library

An updating of phytochemical information on indigenous plants and GLC-HPLC analytical methods is needed. Computer based literature searches should be implemented. Additional journals, especially Journal of Chromatography, should be added to the Library.

h. Record Keeping

A complete record keeping system dealing with instrument maintenance, quality control of sample acquisition, and quality control of analysis procedures is necessary. Reagents, chromatograms, drug, and plant samples need more precise labeling. Some recommended document forms and procedures are listed in the appendices.

i. Instrument Maintenance Program

One or two individuals should receive outside training in routine maintenance of HPLC and GLC, and should carry out a program of regular instrument checks and adjustments. A complete supply of spare parts should be maintained.

j. Operation of GLC and HPLC

Routine use of GLC and HPLC should be limited to a few thoroughly trained individuals. Others should submit samples for analysis to a designated operator.

k. Drug Screening

A phytochemical screening program should be developed in cooperation with a trained animal pharmacology team to evaluate indigenous plants on a large scale.

l. Laboratory Personnel and Training

At least two staff members from the Traditional Medical Centre should be sent abroad for advanced graduate programs in phytochemistry including instrumental analysis.

The Staff Pharmacologist needs specialized training in animal testing procedures.

Two additional staff members should be added and should receive outside training. First, a local graduate of the College of Pharmacy should receive Master's Degree Training in Phytochemistry. This person would serve as a research associate to carry out research designated by current staff members. Second, a suitable candidate should receive outside training in HPLC and GLC operation and repair. This individual would be responsible for running routine analyses and for instrument maintenance and repair.

m. Cooperation With Local Pharmaceutical Companies

Interaction with Keko Pharmaceutical Company and Tanzania Pharmaceutical Company should be expanded. The Traditional Medicine Centre is in an ideal position to provide isolation procedures that could be scaled up to Industrial Production.

2. To The United Nations Industrial Development Organization

a. Provide funds needed for additional capital equipment listed in appendices.

b. Provide funds for disposable supplies and instrument repair for at least five more years.

c. Assist in providing postgraduate education at the doctoral level to three current master's degree staff members and specialized training for two additional staff members at the master's level.

d. Provide an animal pharmacology expert for two years.

e. Provide a phytochemical screening expert well versed in GLC and HPLC separation procedures and quality control. This individual should stay for one to two years.

f. Provide spare parts (unavailable locally) for project vehicle.

III. JOB DESCRIPTION

Two Months - December 14, 1984 to February 14, 1985

Project in the United Republic of Tanzania
DP/URT/81/026/11-03/32.1.D

Purpose of Project: Utilization of the indigenous natural resources of medicinal and aromatic plants for the production of pharmaceuticals.

Duties: The expert will, in collaboration with local counterpart, set up a facility for the chemical analysis of plant material and natural products and recommend appropriate methods of quality control of plant-derived pharmaceutical products. The expert should also train local counterparts in methods of analysis and recommend schemes of further training.

The expert will also be expected to prepare a final report setting out the findings of his mission and his recommendations to the Government for further action which might be taken.

Qualifications: PhD in Organic or Analytical Chemistry or Pharmacology with research experience involving instrumental analytical methods such as GLC, HPLC, IR, UV, NMR.

Language: English.

Background Information:

Tanzania, like many developing countries, has a flourishing traditional system of medicine based on the use of plants as therapeutic agents. Research on both medicinal and aromatic plants are currently being conducted in Tanzania and the Government would like to produce pharmaceuticals from them, for both local use and export.

In 1979, a UNIDO Mobile Unit which visited Tanzania highlighted the potential for the development of a pharmaceutical industry utilizing plant material. The present project is to develop the facilities at the Muhimbili Traditional Medicine Research Unit, attached to the University of Dar-es-Salaam, for the production of pharmaceuticals utilizing medicinal and aromatic plants on an experimental basis.

IV. BODY OF THE REPORT

1. Introduction

This report concerns the current phase of an UNIDO project to assist in developing locally manufactured pharmaceuticals from indigenous plant resources. The project aims include screening uninvestigated Tanzanian plants for possible new therapeutic agents. Earlier phases of the project included identification of therapeutically important local

plants that could be cultivated, introduction of several Romanian plants for cultivation, and finally, construction of a pilot facility for isolation of drugs.

The object of the current phase is to develop a research unit in the Traditional Medicine Centre capable of screening uninvestigated plants for potential drugs, developing isolation procedures for industrial scale-up, and performing quality control analyses on cultivated plants and their derived pharmaceuticals.

The original task of this expert was to develop isolation procedures and train laboratory personnel in quality control using HPLC and GLC instruments.

Upon arrival in Tanzania, it was discovered that the primary analytical instruments were unassembled and critical components were missing. The majority of the mission was spent obtaining needed parts,and identifying long-term instrument and supply needs. Remaining time was spent training personnelin use, quality control, and routine maintenance of the instuments. Following are specific details of the mission.

2. Laboratories

The Traditional Medicine Centre is a two-story building with offices and an instrument laboratory on the first floor. The second floor contains offices, two chemistry laboratories, and animal pharmacology laboratories. Animal housing facilities are in a separate building. The laboratories are clean, well lighted, air conditioned, and contain adequate space for anticipated use. The instrument laboratory has one

door-way to the building interior,and it's only windows face the outside. Enough bench space is available for current instrumentation,but spare parts and file storage cabinets are needed. Air conditioning is adequate, and it is especially important that the dust filter be cleaned regularly. Windows should be sealed on the inside with plastic sheets. A dehumidifier should be installed. Power supply is a special problem. Local current is 240V 50 cycle and is subject to fluctuations as high as 400V. During the tenure of this expert's mission, power cut-offs occurred on an almost daily basis. To prevent instrument damage and provide baseline stability, a heavy-duty voltage stabilizer was ordered. This stabilizer is capable of handling 240V and 120V instruments and can accommodate all major instruments in the laboratory (5 KAV, 220, 240, 120 VAC). However, a voltage stabilizer does not solve the problem of power cut off. A 5 KAV portable generator should be considered for the future,especially if power cutoff continues to be a problem.

3. Instrumentation and Supporting Equipment and Supplies

a. General Support Equipment

The wet chemistr. laboratories contain some equipment needed to carry out isolation and purification, and to support instrumental analysis. This equipment includes drying ovens, evaporators, TLC equipment, a reliable source of distilled deionized water, and a suitable array of volumetric glassware. Some equipment necessary for routine laboratory work is not present, such as a large reliable -20°C freezer and a refrigerator for biological sample storage. Other needed support equipment are a centrifuge, an additional 0.1 mg electronic balance, and a pH meter. A large, low dead volume column for initial isolation of plant components is needed. An extraction unit suitable for liquid/liquid and liquid/solid extraction is needed to develop isolation procedures suitable for scale-up to industrial pilot plant levels. This unit must be large enough to handle 2 to 3 kg of material and, therefore, enable handling of plant material in amounts necessary for isolation of chemical constituents.

b. Supporting Supplies for Varian 3700 GLC

At the time of this expert's arrival, all components necessary for assembly and start-up were present except for gases, regulators, and electrical connections. During the course of the mission, sources were identified for necessary gases. Enough gases and equipment were temporarily borrowed to start up and check out the instrument. Breathing air of suitable quality is available locally(Tanzania Oxygen Supply). A short-term supply of carrier

gas (Argon) is available locally, but long-term supplies will have to be obtained externally (Nitrogen). Local users of GLC equipment have used the following suppliers for gases:

Murex Welding Products
Gas Control Systems
Lea Road Waltham Abbey
Essex England 1 AU
UK

Telex 299163

Supporting disposable supplies needed for one to two years operation were ordered. A reliable supply of gases is necessary. They should be purchased on a regular basis allowing two months lead time to prevent shortages. Solvents needed for sample preparation will also need to be purchased on a regular basis.

c. Supporting Supplies for HPLC

Several critical components and all technical manuals were missing from the HPLC as received. A list of missing manuals and parts was telexed to the supplier in December 1984, but no reply was received by mid-February 1985. Consequently, the instrument cannot be assembled until parts arrive. Ancillary supporting supplies have been identified and are listed in Appendix 2.

HPLC systems require extremely high purity solvents on a regular basis. A source for these solvents has been identified (BDA Chemicals, Poole, England) and an initial order has been placed. HPLC columns will need to be replaced on a routine basis. HPLC systems also require water of extremely high purity. This water can be purchased, but a more economical solution would be to

purchase an HPLC water purification system (Milliport, Inc, Milli-Q System, approximately $1500).

d. Supporting Supplies for IR

The infrared instrument was assembled and several test spectra were generated.The insrument functioned within specifications. Needed supplies are a pellet press and sample cells(liquid and solid). Day-to-day costs of running an infrared instrument are minimal. Disposable supplies needed on a regular basis are inexpensive salts for pellet preparation.

e. Sources of Supply for Capital Equipment

Special problems occur when an instrument laboratory is established and maintained in a developing country. Routine maintenance of equipment and acquisition of supplies are orders of magnitude more difficult than in the United States. This expert strongly recommends that gas liquid chromatographs (GLC) and high performance liquid chromatographs (HPLC) should be purchased from suppliers who specialize in such equipment and deliver complete turn-key packages. In addition the suppliers should have a strong track record of maintenance and methods development support.

In the current project, Varian associates supplied an essentially complete GLC instrument with all needed tools, spare parts, and manuals. Varian also provided excellent methods development support.

The HPLC was supplied by a distributor that does not specialize in HPLC instrumentation. The instrument was supplied incomplete and without technical manuals. Great difficulty was experienced in obtaining critical components, none of which arrived during the expert's mission.

4. Access to Information

Modern instrumentation and training are not enough to maintain an effective research laboratory. Access to current literature and contact with established research groups are necessary to produce quality results. Journals related to phytochemical screening and assay development should be added to the Muhimbili College of Pharmacy library. Journal of Chromatography, Phytochemistry, and Journal of Natural Products, are recommended. Access to a literature search service such as NAPRALERT (University of Illinois, Dr Norman Farnsworth) would reduce chances of repeating work of others, provide latest methods of screening and analysis, and enable laboratory personnel to remain current with their international peers. Frequent contact with research scientists in one or two universities in Europe and the United States would provide invaluable exchange of ideas, techniques, and experimental results.

5. Training

a. General Comments on Training

The Traditional Medicine Centre is equipped with facilities and instrumentation necessary for its goal of becoming a major research laboratory. However, laboratory personnel do not possess the experience and training needed to effectively utilize the instrumentation. Further training is absolutely necessary in the following areas:

1) Animal pharmacology
2) Use of GLC and HPLC in phytochemical screening
3) Quantitative analysis and quality control procedures
4) Instrument maintenance

This training can be accomplished by sending laboratory personnel to European or American laboratories for advanced training and by sending outside experts to the Traditional Medicine Centre. A combination of both would appear to be the most effective alternative. The presence of technical experts would allow the laboratory to function while key personnel are engaged in training elsewhere. Detailed recommendations for training appear below and in Section 6. This expert recommends hiring at least one additional bachelor's level staff member. This individual should receive outside training in HPLC and GLC maintenance and be responsible for instrument maintenance and routine analyses.

b. Animal Pharmacology

Pharmacological effect screening is indispensable for identification of potential plant derived medicinals. One individual at the Muhimbili Centre is responsible for pharmacologic screening in animals. He is a clinical pharmacologist and needs outside training in animal research techniques. This expert recommends adding an additional staff member at the master's level to assist in conducting studies.

c. Phytochemical Screening

Several individuals at the Muhimbili Center have limited experience in phytochemical screening but do not have training in modern instrumental techniques. This expert recommends sending at least two faculty members abroad for graduate training in phytochemistry.

d. Quality Control Procedures

Quality control in the research laboratory depends on developing and adhering to standard operating procedures(SOP). These SOP must be thoroughly documented and complete records should be kept of sample acquisition, sample storage, pharmacological screening, chemical extraction, and quantitative analysis. Quality control standards of essential oils and other extracts should be stored in a -20°C freezer and regularly checked for purity. Standard operating procedures must be established for routine calibration and maintenance of laboratory instrumentation, especially electronic balances, GLC, and HPLC equipment.

The course of the current mission was too short to adequately train laboratory personnel and to implement quality control procedures. Further training is necessary both by sending experts to Tanzania and by sending Muhimbili personnel to outside laboratories for work experience.

A supply of quality control standards must be maintained and regularly checked for purity. When not available from overseas sources, they may be prepared by local chemists.

Some suggested quality control documentation is listed in Appendix 3.

e. Instrument Maintenance

This expert visited several laboratories in Dar-es-Salaam. A significant number of analytical instruments were not functioning in every laboratory visited. Discussions with laboratory managers concerning reasons for instrument malfunction led to the following conclusions:

1) Fluctuations and interruption of electrical power is a major source of instrument burn-out. Damage from power fluctuations can be minimized with voltage stabilizers.

2) Heat, humidity, and dust are major contributors to electronic instrument failure in the Dar-es-Salaam area. These problems can be minimized by placing instruments in rooms that are effectively sealed (ie, plastic over windows), air conditioned, and dehumidified. This expert visited only one

laboratory (Tirdo Instrument Repair) that provided voltage stabilization, air conditioning, and effective dust exclusion.

3) Absence of replacement parts, absence of personnel trained in maintenance, and failure to develop calibration and routine maintenance procedures contributes heavily to instrument failure.

The Muhimbili Traditional Medicine Centre has initiated steps to solve the instrument maintenance problems listed above. The laboratory is moderately isolated from dust, and steps are being taken to seal outside windows. Air conditioning is adequate and a voltage stabilizer has been ordered. This expert also recommends purchase of a dehumidifier.

Training in instrument maintenance was initiated by this expert, but time was too short to provide a thorough briefing. It is vitally important that this training continue. This expert recommends a combination of sending laboratory personnel to outside laboratories and bringing quality control experts in to the Traditional Medicine Centre.

4) Work plan for the Traditional Medicine Centre

a) General comments

The Muhimbili research centre is in an ideal position to perform the following functions:

1. Screen the more than 1000 uninvestigated medicinal plants of Tanzania for effective therapeutic agents.

2. Provide bench scale purification procedures that can be scaled up in the local Keko Pharmaceutical Co. pilot plant.

3. Provide quality control checks on locally extracted pharmaceuticals and essential oils.

4. Provide quality control data to optimize yields from cultivated plants, such as time of harvest, cultivation conditions, and selection of appropriate species.

5. Serve as a general scientific resource and communications link between Keko Pharmaceutical Co.(Dar es Salaam) and Tanzania Pharmaceutical Industries Ltd (Arusha).

b) Plant derived medicinals.

Implementation of the recommendations contained in this report would allow pharmacological screening, extraction, purification, identification and manufacturing scale up of components from approximately 20 plant species per year. The laboratory could achieve this output within two years of training implementation.

c) Training of personnel.

In summary, the following training is recommended:

1. Send two staff members abroad for advanced graduate programs in phytochemical screening.

2. Send the staff animal pharmacologist abroad for specialized training in animal screening.

3. Two additional staff members should be recruited locally and should receive outside training. First, a local graduate of the College of Pharmacy should receive Master's Degree training in phytochemistry. This person would serve as a research associate to carry out research designated by current staff members. Second, a suitable candidate should receive outside training in HPLC and GLC operation and repair. This individual would be responsible for running routine analyses and for instrument maintenance and repair.

4. Bring an outside animal screening expert to the Traditional Medicine Centre for a minimum of one year.

5. Bring an outside phytochemist expert to the Traditional Medicine Centre for one to two years.

APPENDIX 1

List of Officials and Experts Encountered During Mission

1. Mr. S. K. Henein
   SIDFA

2. Mr. Erling Skjonsberg
   JPO

3. Mr. E. N. Mshiu
   Director, Traditional Medicine Research Centre (TMRC)

4. MR. E. S. N. Shunda
   Forestry research activities

5. Dr. Makenne
   Dean, Muhimbili Medical Training Centre, University of Dar es Salaam

6. Dr. Konje
   Head, Pharmaceutical Division, College of Pharmacy, University of Dar-es-Salaam

7. Dr. S. C. Chabra
   TMRC

8. Mr. E. B. Katanga
   Production Manager Keko Pharmaceutical Co.

9. Dr. E. Njau
   General Manager, Tanzania Pharmaceutical Industries Ltd.

10. Mrs. Uiso
    TMRC

11. Mr. Kayungi
    TMRC

12. Mr. Mhiso
    TMRC

13. Mr. J. Dorsey
    Professor, College of Pharmacy, University of Dar-es-Salaam

APPENDIX 2

Anticipated Capital Equipment & Disposable Supply Needs

1. Capital equipment

   a. 0.1 mg electronic balance (~$1000)

   b. -20°C freezer (~$400)

   c. 4°C refrigerator (~$400)

   d. 2000 rpm centrifuge (~$1000)

   e. 2 to 3 kg bench scale extraction unit (~$600)

   f. Millipore water purification system (~$1500)

   g. pH meter(~$500)

2. Disposable supplies

   GLC gases, HPLC solvents, various reagents, and disposable equipment are needed for bench scale extraction and analysis. Anticipate costs per year are approximately $3000.

APPENDIX 3

Quality Control Documentation

Failure to document maintenance and analysis procedures will ruin valuable laboratory work.
Documentation must include analysis, maintenance, and troubleshooting procedures.

The following examples of documentation have proven effective in other laboratories and may serve as general guides for the Traditional Medicine Centre.
They are partially reproduced from **Maintaining and Troubleshooting HPLC Systems** by D.J. Runser and from UNDP Report DP/NEP/80/003 authored by Jan Karlsen.

A. Recommendations for Primary Record Keeping during the Examination of Plant Materials for Biological Activity

It is assumed that a research group consisting of a project director, a field botanist, a chemist*, a pharmacologist, and a microbiologist has been established.
It is further assumed that a series of objectives (specific plants to be examined) has been established and that a program of plant collection relating priority to availability has also been established.
It is desirable that a numerical target is set for the group.
This target will be dependent on the facilities available, eg, plants, number of extractors, availability of enough animals, and sufficient staff.
It is also highly desirable that the research group should meet frequently and formally (ie, that all members should be present at a specified place and time).

It is anticipated that the project director will keep a file for each plant species which will include an indication of the reasons for the choice of the particular species, the types of activities which have been reported, and a few key references.
These data will be of use when evaluating the plant and planning any future action.

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*This chemist should have special experience within the field of analytical chemistry to offer assistance and advice in questions concerning quality control.

Plant Collection Form to be Filled in by Field Collection as far as Possible IN THE FIELD

1. Plant name: _____ Tanzania name: _____

2. Plant part: _____ 3. Species no. _____

4. Collection no. _____ 5. Date collected: _____

6. Voucher specimen no.: F/SSS/CC/DD,DD/DD/V/P

7. Site of collection:

8. Condition of plant when collected:

9. Condition of field storage:

10. Amount of material available at collection site:

11. Comments:

12. Field notebook ref:

13. Plant collected by: _____ Signature _____

14. Plant delivered to: _____ Signature _____

15. Date of delivery: _____

Copies to:

Project Director
Chemist
Pharmacologist
Microbiologist

Bioassay of Plant Material

The bioassay results may be negative, positive, or equivocal.

If the results are equivocal, then a higher dose range, a different route of administration, or a different should be attempted.
vehicle.

If the results are negative, then no further action need be taken; however, there may be reasons to believe that the negative test is not a good representation of the plant's activity and a higher dose range may be attempted.

If the results are positive, the experiment should be repeated to verify reproducibility of activity.

Thus, given any result, it could be argued that a second collection should be made at a different time of the year and the activity of that extract compared with the first.

NOTE: Certain modifications have been suggested:

1. Specimen number SSS not be included.

2. V and P not be included.

3. That the ethnic group using the "Tanzania" name be included.

4. That when possible, photographs of the material be made and a question. Photograph: Y, N be added to the Field Collection form.

Bioassay 1 - Pharmacological Testing

1. Plant name: _____ Tanzania name: _____

2. Plant part: _____ 3. Species no.: _____

4. Collection no.: _____ 5. Date collected: _____

6. Extract no.: B/SSS/CC/DD,DD/DD/EE/AA

7. Date received: _____ 8. Dry weight equivalent: _____

9. Solubility/Vehicle: _____

10. Reported activity: _____

11. Appearance: _____ 12. LD_{50} _____

13. Assay type: _____ 14. Date commenced: _____

15. Active Y, N, E: _____ 15. ED_{50} _____

17. Comments (record or refer to any other bioassay and toxicity data):

18. Notebook no., page no.: _____

19. Carried out by: _____ Signature _____

20. Date: _____

21. Comment and recommendation by Senior Pharmacologist:

Copies to:

Project Director
Botanist
Chemist
Microbiologist

Bioassay 2 - Microbiological Testing

1. Plant name: _____ Tanzania name: _____

2. Plant part: _____ 3. Species no.: _____

4. Collection no.: _____ 5. Date collected: _____

6. Extract no.: M/SSS/CC/DD,DD/DD/EE/TT

7. Date received: _____ 8. Dry weight equivalent: _____

9. Solubility/Vehicle: _____

10. Reported activity: _____

11. Appearance: _____

12. Test type: _____ 13. Date commenced: _____

13. Active Y, N, E: _____

15. Comments:

16. Notebook no. 1, page no.: _____

17. Carried out by: _____ Date _____ Signature _____

18. Comments and recommendation by Senior Microbiologist:

Copies to:

Project Director
Botanist
Chemist
Pharmacologist

Key to Form

Form P1. Field Collection

1. Plant name, both binomial and local.

2. Plant part.

3. Species number. This should be allocated from the master list of plants. SSS

4. Collection number. This is allocated by the botanist in the field and refers to his collection. This should also be recorded in the field notebook.

5. Date collected. The date the specimen was collected, **not** the dates of the collecting trip.

6. Voucher number.

   F=Field collection, SSS from 3, CC from 4, DD,DD,DD date from 5.
   V=Voucher specimen, P is the place at which the voucher specimen is lodged.

7. Site of collection.

   This should be described in sufficient detail to allow a recollection of a sample of the **same** population from the **same** site, ie, was it in heavy shade, on N or S facing slopes, etc.

8. Condition of plant. Was the plant in bud, in full flower, etc? Was the plant infested with fungi or ants, etc? Had the plant been damaged by browsing animals?

9. Condition of field storage (burlap bags, plastic, loose, bottom, or top of large pile of material).

10. Amount available at collection site. Was the plant abundant or sparse?

11. Comments. Can be used to expand 7-10.

12. Field notebook reference, page number.

13. Name, (printed) and signature of collector.
14.

15. Name, (printed) and signature of person taking over the plant from
16. the field collector.

17. Date plant material passed to next person (presumably the chemist who is responsible for extraction).

Form P2 - Chemical Extraction Form

1-5 as P1.

6. Specimen number C indicates chemistry, SSS/CC/DD,DD,DD as P1. EE Extraction number allocated by chemist.

7. Date received by chemist.

8. Condition of material on date of extraction.

9. Drying method.

10. Weight of dried plant. If only part of the sample is used for extraction, then this should be indicated here and that figure used in the estimated of the Dry Weight Equivalent (19).

11-12 Dates extraction commenced and completed.

13-15 Conditions of extraction.

16. Has the extract been deionized? Yes or No

17. Concentration procedure.

18. Residual Weight.

19. DWE. This is the yield of extract from one kilogram of dry plant (18/10 g/kg). (For example, if 500 grams of dried plant gives rise to 150 grams, then DWE = 300 g/kg. If 1.4 kg of dried plant gives rise to 15 grams, then DWE = 10.7 g/kg.

20. Aqueous solubility. Yes or No

21. Appearance

22. Amount sent for bioassay

23. Date sent

24. Amount sent for microbiological testing

25. Date sent

26. Comments

27. Notebook number and page number

28-29 Name and signature of chemist

30-31 Name and signature of pharmacologist and microbiologist

32. Date material passed to pharmacologist and microbiologist

Form P3

1-5 as P1

6. Extract number B=Bioassay, SSS,CC,D,D,D,D,DD as P1,2. EE extract number as P2, AA bioassay number.

7. Date received from chemist.

8. Dry weight equivalent from chemist's form.

9. Solubility/Vehicle (if not soluble in water, vehicle should be recorded here).

10. Activity reported in literature, eg, anthelminthic.

11. Appearance of extract at time of bioassay.

12. LD_{50}.

13. Assay type performed, eg, antidysentery.

14. Date assay commenced.

15. Active. Yes, No, Equivocal

16. If active, ED_{50}.

17. Comments. Refer here to any other bioassay and toxicity data.

18. Notebook reference number, page number.

19-20 Name, signature of person who carried out assay and date.

21. Comment and recommendation by senior pharmacologist.

Form P4

1-5 as P1

6. Extract number M=Microbiological test SSS/CC/DD,DD,DD as P1,2,3. EE extract number as P2. TT microbiological test number.

7. Date received from chemist.

8. Dry weight equivalent from chemist's form.

9. Solubility/Vehicle (if not soluble in water, vehicle should be recorded here).

10. Activity reported in literature.

11. Appearance of extract at time of microbiological testing.

12. Test type performed, eg, antibacterial - E. coli.

13. Date assay commenced.

14. Active. Yes, No, Equivocal

15. Comments.

16. Notebook reference number, page number.

17. Name, signature of person who carried out assay and date.

18. Comment and recommendation by senior microbiologist.

B. HPLC Operations Manual

This is a collection of policies and procedures encompassing analysis, maintenance and troubleshooting.

1. Floor plan of laboratory and location of instruments and parts.

2. Inventory of all components and supplies.

    a. Name
    b. Serial number
    c. Location in lab
    d. Manufacturer
    e. Supplier
    f. Purchase date
    g. Replacement cost
    h. Location of manuals
    i. Location of maintenance logbooks
    j. Technical service representative name, company, address, telephone number, and telex

3. Inventory of all HPLC columns

    a. Name Identification number
    b. Location
    c. Manufacturer
    d. Supplier
    e. Cost
    f. Location of column logbook

4. Laboratory and equipment start up and shut down

5. Care of equipment

    a. Column labeling procedure
    b. Use of logbooks
    c. Use of swage lock fittings

6. Mobile phase preparation

    a. Solvent use
    b. Care and labeling of solvent containers

7. Sample preparation

    a. Clean up

8. Column cleaning procedures

9. Cleaning and repairing injector

10. Quantitative measurement techniques and calculations

11. Recording data

12. Internal contacts and responsibility list with addresses and phone numbers

    a. Person in charge
    b. Lab assignments

13. HPLC reports, forms, and labels

    a. When they are used
    b. How to fill out
    c. Examples of each

14. Reference texts

C. Labels for Equipment and Solvents

1. Solvent preparation equipment. Label solvents used or to be used with specific equipment.

2. Solvent reservoir.

   a. Mobile phase solvents

   b. Concentration

   c. Date prepared

3. Solvent delivery systems

   a. Pump heads as to last date seals, plungers, and so on, were changed

   b. Date last used

   c. Solvent last used

4. Sample introduction

   a. Type of septum in the injector

   b. Solvent last used in the injector

   c. Pressure limit of the injector

   d. Date of last rotor change

5. Columns

Inlet/outlet Packing material Solvent last used Storage solvent if difference from c Date last used Detector. Date source lamp was changed.

D. HPLC Assay Report

Number _____

Date _____

Analyst _____

Notebook _____ Page _____

HIGH PRESSURE LIQUID CHROMATOGRAPHY REPORT

Objective _____

Substance _____

Product-Formulation _____

_____

Instrument used _____

Column Liquid phase _____ Support _____

Length _____ Diameter _____ Material _____

Temperatures: Column _____°C

Eluent _____

Flow rate _____ ml/min at _____ PSI

Concentration: _____ mg in _____ ml of _____

Injection Device _____ volume mcl equivalent to _____ mcg

Derivatization _____

Detection Type _____ Range _____ Attenuation _____

Type _____ Range _____ Attenuation _____

Retention times _____

Results and Comments: (linearity range, limit of detection, stability, etc)

E. Routine Laboratory Tasks

1. Maintain the fittings, hardware, tools, and spare parts inventory.

2. Maintain adequate inventories of HPLC solvents.

3. Monitor the inventory, usage, and handling of HPLC columns.

4. Monitor the updating of the operations manuals, calibration and maintenance logbook(s), and troubleshooting logbook(s).

5. Maintain the supply of instrument manuals.

6. Repair worn sampling values.

7. Pack and quality check columns.

8. Perform routine maintenance on pumps, detectors, and recorders.

F. GLC Record Keeping

Documents for GLC use are prepared in the same format as for HPLC.

APPENDIX 4

List of Spare Parts Needed for Landrover

-- Year of Engine - 1983

-- Manufacturers - Rover Company

-- Chassis No. - 195347

-- Engine No. - 36132269 (B)

No. of Units	Name	Approximate Total Price (US Dollars)
1	Water Pump Kit RTC 3072	60
1	Crankshaft Oil Seal UKC 467	10
1	Clutch Master Cylinder Kit (610611)	10
4	Universal Joints GUJ 117	40
4	Hub Seals GHS 202	40
2	Pinion Oil Seals AEU 2515	20
1	Steering Column Bearing RTC 324	10
1	Salve Cylinder Kit 8G 8600	4
2	Swivel Pin Seals GHS 1003	10
1	Brake Master Cylinder Kit (606415)	12
1	Carburetor Overhaul Kit (605092	60
2	Set Points GCS 125	14
8	Spark Plugs GSP 131	24
1	Set Starter Brushes GSB 112	10
1	Flasher Unit GFU 124	12
3	Tie Rod Ends GSJ 137	
3	Tie Rod Ends GSJ 153	
2	Output Oil Seals FRC 1780	16
2	Fan Belts GFB 124	10
	Total 10% Transport Grand Total	542 55 597