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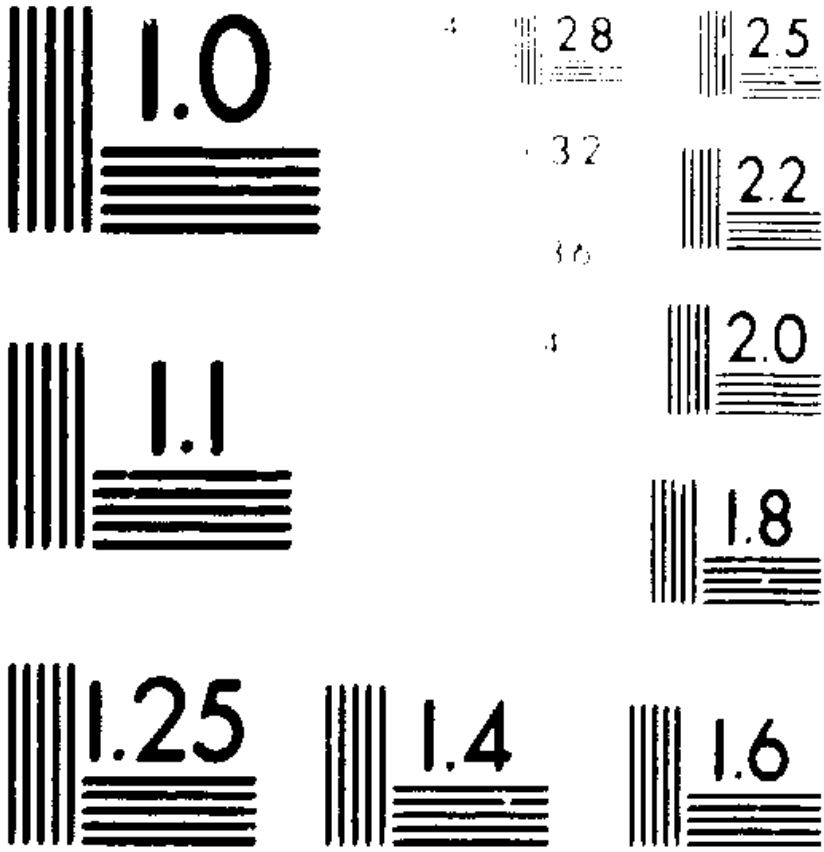

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Nepal

STRENGTHENING THE ROYAL DRUGS RESEARCH LABORATORY

DP/NEP/80/003

NEPAL

Terminal report *

Prepared for His Majesty's Government of Nepal
by the United Nations Industrial Development Organization,
acting as executing agency for the United Nations Development Organization

Based on the work of Jan Karlsen,
expert in analytical chemistry/quality control

United Nations Industrial Development Organization
Vienna

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I. SUMMARY

The basis of this report is concerned with the aspects of the instrumental equipment at the RDRL, bringing it up to a position where the instrumentation meet modern requirements for a research laboratory. Secondly the setting up of a working programme to be continued in the coming years for the screening of plant material which have been selected for investigation was of importance.

The conditions in Nepal are favourable for the starting of small scale production of plant products. However, rigorous standard methods for the control of the products must be developed and applied if these products are to achieve a market. A UNIDO expert in economic mapping has started a survey of Nepal and his investigation will naturally be the basis for the plants selected for cultivation or collection. However, it is my opinion that RDRL should carry on with the collection of the essential plants from all over Nepal to establish a Nepalese standards (i.e. Cortex Granati) now that the necessary instrumentation for phytochemical work is present. However, whatever rich the flora of Nepal may be, the amount of work necessary to establish own standards must not be underestimated.

The biological departments of the RDRL are the weakest and most in need of future strengthening. Therefore some of the instruments have been chosen deliberately to enable the chemical departments either to provide the section of pharmacology with purified extracts or "pure" compounds - or to analyse more thoroughly any extract showing positive pharmacological effects.

To replace imported pharmaceutical products with indigenous drugs require extensive pharmaceutical/pharmacological testing without which no success can or will be achieved. It is therefore stressed that the development and production of medicine based on indigenous material is a long and tedious process. RDRL being the key laboratory in this the development in Nepal certainly will need long term support to achieve the goals set by HMG.

Specifically is also mentioned the necessity of a cooperation with other universities concerned with research in phytochemistry. Such a contact could lead to a joint Ph.D. programme and facilitate the exchange of Ph.D. students.

To enable Nepal to enter the market of perfumery chemicals a number of essential oils are investigated. Total oils or "cuts" thereof may be successful products for Nepal in the future. The programmed harvesting - distillation and analysis being done at present will be carried out into 1985 and should provide RDRL with necessary data for product evaluation. This programme will be extended to other plants containing essential oils. Instrumentation and methods for essential oil control has been established, essential oil standards have been evaluated and a good start in the field of essential oil evaluation achieved.

In the field of pharmaceutical drug control the instrumentation provided should be sufficient for efficient control, however, post-graduate experience from other drug control laboratories is urgently needed for the officers. Further-more support from UN-experts is also strongly recommended because the concept of state drug control is depending upon international cooperation to be of any use to the country it is applied. It is therefore necessary and recommended that regional cooperation between Nepal and Sri Lanka, Bhutan, Bangladesh, Burma, Thailand, India, Singapore, Malaysia is established on a continuous basis. This cooperation does not only concern the aspect of drug quality control, but should also include the establishment of small manufacturing units based on indigenous plant material.

Concern is given to the necessity of having a regular supply of chemicals, vector gases and continuous supply electricity without which the work being done will turn out to be extremely inefficient.

The regular meetings of staff (every week) which are initiated to discuss problems occurred will further strengthen the cooperation between sections.

It is the opinion of the expert that RDRL has been considerably strengthened due to the input of instrumentation and to the discussions with RDRL's officers on the planning of research activity. As pointed out research in the field of drugs and drug production is a long-term undertaking and need therefore long term support. Observing the need for Nepal to develop production facilities for cheaper drugs this must be considered a laudable project.

An increase in the speed by which the Royal Drugs Research Laboratory turns into a modern drug research laboratory largely depends upon a continued support by the UN agencies. As pointed out during numerous discussions the developing of acceptable drugs is never a short term project, but takes many years to realize. The expert will therefore most strongly recommend more support to be given to RDRL.

II. RECOMMENDATIONS

1. HMG of Nepal has decided to start a programme to make the country self-reliant of the "essential drugs" in the future. Such a programme, which must be supported, is a long term project which will require extensive support by the United Nations in the form of expert assistance, purchase of equipment as well as support for post-graduate studies.

2. The analytical department/quality control must be further strengthened regarding the analysis of drug standards. More training in projects, where a close cooperation between the pharmacological and analytical department is required, is necessary.

3. The close cooperation between herbal farms and the central laboratory should be continued as this is necessary for the evaluation of the essential oil production.

4. All efforts must be concentrated on the problem of continuous energy supply without which the work in an analytical laboratory becomes very difficult.

5. A supply line for vector gases and essential chemicals should be established on a regular basis to overcome the difficulties when RDRL run out of some essential items. A Calcutta-Kathmandu line seems the most obvious solution.

6. Other essential oil standards ought to be procured from the main international companies dealing with perfumery raw materials.

7. The standards, which have to be kept in the refrigerator, ought to be controlled regularly by GLC-analysis.

8. Closer cooperation with one or two universities in Europe is recommended for the exchange of results and ideas concerning research and post-graduate education.

9. An updating of the literature available in Nepal on indigenous medicinal herbs is strongly recommended. A computer-based literature search should be carried out. A revised list of medicinal herbs should be made.

10. Extend the contacts with other drug control laboratories in South East Asia. This is most essential for the department of Drug Quality Control.

11. Maintain a stock of "essential drugs" at the EDRL and continuously check their purity.

12. Train some of the officers in instrument maintenance. More than one person must have this as his/her responsibility.

13. Continue to keep the analytical result on the computer file to allow easy access to previous result on drug analysis.

14. Keep records on the usage of solvents for the HPLC as this will change the purchase pattern of solvents considerably.

15. Encourage the officers to have weekly meetings to discuss problems regarding their research projects.

16. Make a programme for regular maintenance of the instruments.

III. JOB DESCRIPTION

To develop further the existing facilities for the production of plant derived pharmaceuticals at the Royal Drugs Research Laboratory Kathmandu/Nepal.

The expert analytical chemistry/quality control will be expected to conduct quality assessment on pharmaceutical products.

Specifically he will develop analytical methods for determining the content of active ingredients in medicinal and aromatic plants and all products prepared from these plants.

IV. BODY OF THE REPORT

A. General Remarks

1. It is the policy of HMG to make Nepal self-reliant of essential drugs. The foreign exchange resources does not permit the acquisition the basic needs of these essential drugs. One way in which this situation can be met is in the development of pharmaceuticals derived from plants used in the traditional systems of medicine.

2. To utilize plant derived pharmaceuticals or chemical products the standardization of procedures and techniques in the preparation of such extracts would be a first step. In order to ensure the wide acceptance of extracts by health authorities in both developing and developed countries it would be necessary to ensure the following:

a. Authenticated plant material of uniform quality

b. Strict conformity to prescribed/predertermined methods of extraction

c. Rigid control of quality during all stages of production and in the final products.

These point must also be stressed when research on plant material/plant products is carried out.

3. The Royal Drugs Research Laboratory has a split function - one is to carry out research into the usage of indiginous plant for drug production and essential oil production - the second one is to act as the national drug control labor tory. For these two operations adequate facilities regarding, manpower, library, instrumentation and back-up of technical maintenance are necessary. My operation therefore started up as summing-up of the status of the RDRL regarding analytical equipment and discussions with counterparts and technical experts as to how we best could improve the facilities. With this aspect in mind the list of equipment was changed to better suit the immediate problems to be solved in RDRL (February 1983).

Since the functions of the RDRL must cover research and national drug control it is important that the investment in instrumentation should cover equipment to be used for both purposes.

Before returning to the instrumentation I inspected the localities for analytical, instrumental analysis. Three large rooms has been set aside for this section. Two of the rooms acting as a laboratory and the third one being used as a temporary store. In view of the fact that larger extra analytical instrumentation would be placed on order a repainting and dividing of the third room was discussed with Dr. S.R. Adhikari and Dr. S.B. Malla. As soon as the Nepalese fiscal year would start this third room would be made ready for new instrumentation (July 1983). As instruments like computers and Fourier Transform Infrared requires reasonable dustfree surroundings it is important that at least one room be kept at that standard.

The third room was then divided into two whereby one part was to be the place to put the microcomputer and the Fourier Transform Microprocessor, the other was intended to be storage for spare parts for the larger instruments. The three rooms are all equipped with air-conditioners to enhance the stability of the analytical instruments.

The section of instrumental analysis was also carrying out simple measurements like pH det. and routine spectrophotometric analysis required by the section quality control/public analysis. After discussion with Dr. S.R. Adhikari and Dr. Malla it was decided to divide one of the laboratories on the ground floor to make it suitable for these routine analyses and that this room was to be used by the section public analysis/quality control.

Being a landlocked country with transportation problems it is obvious th t the running of a research laboratory will create problems. Regular supply of vector gases and chemicals is crucial to a continuous operation of the laboratory.

Even with the best of planning it is inescapable that a shortage of supply of certain chemicals will occur. It is therefore of utmost importance that a supply line from India should be maintained. This supply line would allow RDRL to get chemicals within 2-3 weeks in considered necessary by the expert.

At the same time a running of the store in a more efficient way will also become a necessity. To assist the administration a micro-computer which would generate stock lists would be necessary.

Another aspect considered under the heading general precautions is the continuous supply of electricity (220 V, 50Hz). In the spring 1983 only some breaks during the working hours occured and the problem was not considered too serious for the output of the results. However, during the pre-monsoon and the monsoon period, the situation became worse and irregular breaks in electricity every day was experienced. This kind of irregular power supply is disastrous to instrumental analysis. It seems absolutely necessary to have a generator as a power back-up. Two generators have been ordered, however the one intended for the instrumental section has to be modified to be suitable for 220 V, 50Hz. This has been discussed with the CTA.

B. Analytical Laboratory

Through discussions with the counterpart, a survey of the laboratory facilities and discussions with the technical expert of pharmacology the following equipments were recommended for strengthening the laboratory.

1. All round extraction unit, suitable for liquid/liquid and liquid/solid extraction. This unit must be large enough to handle 2-3 kg(s) material/liquid. This unit is supposed to be the next scaling-up from the amounts of plant material used for screening at bench level. It will also enable the handling of plant material in amounts necessary for the isolation of chemical constituents.

FPO - 004875
Extractor 11/EX H2
(J. Bibby Science Products Ltd.)

2. A distillation unit large enough to scale-up the bench-level experiments for semiproduction scale testing. Enough essential oil should be distilled in this unit to allow distillation time/yield measurements. This unit should further allow fractionation or cutting of the essential oil.

UNIDO Purchase Order - 15-3-H0490
2 Distillation units (20 l.)
(Karl Kolb GmbH & Co. KG)

3. The plant material to be screened and further investigated will be collected in the wild by officers of the RDRL or bought by them in the local markets. It is, however, an absolute necessity for any phytochemical investigation that reference plant material be kept at the Botanical Garden at Godavari and that a central record keeping system be followed up at the RDRL. Any initial investigation of herbal material must start with authentic material i.e. material collected fresh. I therefore recommend the same record keeping procedure as proposed by the technical expert pharmacology. For this record-keeping the RDRL may use modern facilities as a computer. However the technical expert analytical chemistry feels that the officers of the RDRL should first be trained in the usage of micro-processors (personal computers) to realize the advantage of computer aid. A micro-processor will also be applicable to other fields concerned (quality control record keeping, statistical calculations and general computer training). After discussion with counterpart and the technical expert pharmacology, the purchase of a second APPLE computer was recommended.

APPLE II(e)
Epson MX 80
Corvex Hard Disk.

4. Any research laboratory needs a good library. The books and journals presently available at the RDRL cover the field of analytical chemistry reasonably well. The easy availability of a copy-service from a larger library is however lacking. The service given to RDRL on requests of copies from other research institutions has been slow and often fails. It may be anticipated that the improvement we have seen in the postal services for Nepal during the last year may have its impact on the communication with other libraries. I found it necessary to recommend the purchase of some new books in analytical chemistry and perfumery science.

(List of book among the annexes).

5. Vector gases for gas liquid chromatography. Upon arrival in January it was found that no gases were available although an order had been placed in September 1982 for a supply from India. Having no experience with the easy or difficult communications with India no immediate action was taken. No emergency would occur until later in

the year when harvesting/distillation time would start. However as no gases had appeared in the beginning of April plans were made for a direct supply link with Calcutta.

One of our landcruisers were fitted out for transportation of gas cylinders and the UNDP office in Calcutta given due warning of our arrival. Six empty cylinders were brought down to Calcutta. The following documents had to follow the cylinders:

a. Statement of ownership of cylinders and an official order for the filling with special gases

b. Documents showing where the cylinders were manufactured

c. Document from the Department of Explosives in India showing the allowed pressure or that the cylinders comply with the general specifications for gas cylinders.

Hydrogen gas is not available at short notice in Calcutta since this gas is only transported from Bombay once a month. However, authorities at the Indian Oxygen Company promised us the gases in about four weeks time after notifying. After six weeks the cylinders were ready for shipping from Calcutta. I consider this information sufficient to conclude that with proper planning gas can be brought up from Calcutta within a week. This gas transport can easily be combined with the purchase of chemicals as these usually can be bought off the shelf.

6. Following up on the extraction units purchased, equipment is necessary for column chromatography. With this equipment crude extracts can be separated into fractions or single components. Any research laboratory has a constant need of reference compounds. Instrumentation for low pressure liquid chromatography is therefore strongly recommended for the preparation of these compounds. This kind of equipment is also very much applicable to the separation of a crude plant extract prior to biological screening. The low-pressure liquid chromatographic equipment has a fraction collector to enable automatic continuous operation outside the usual working hours. A separation and collection of fractions of crude plant extracts is an operation that very often take a long time.

Multirac Fraction Collector (LKB)
LOBAR chromatographic columns (E.Merck)
DURAMAT dosing pump (Chemie und Filter AG).

7. Equipment for High Performance Liquid Chromatography is a necessity for any research laboratory dealing with drug analysis whether it be governmental control tasks or basic research. Preferably the instrument should be equipped with a UV-detector and a refractive index detector. Additional facilities for gradient elution is not strictly necessary and should be added at a later time when enough proficiency in handling the HPLC-instrument has been acquired. The HPLC equipment should allow for analytical as well as preparative separations. What is important for RDRL, Nepal is the possibility of quick, efficient maintenance service when needed. We therefore settled for a Singapore based company with regular service trips to India, Burma and Nepal.

High Pressure Liquid Chromatograph
(Waters Associates Pvt. Ltd., Singapore).

C. Distillation of Essential Oils

For the investigation of the indigenous essential oils of Nepal once the primary screening has taken place at the RDRL a larger scale investigation of plant propagation must take place in the herbal farms. This prompted a visit by the technical expert - analytical chemist to Tarhara, Hetauda, Tistung and Daman to inspect the distillation procedures. With the experience of the officers at the herbal farms, suitable routines for harvesting and distillation of oils are applied following the general requirements:

1. Plants must be collected following a programme and the data necessary for quality evaluation filled in on all forms.

2. Technical data concerning the distillation procedures must also be written on the above mentioned forms.

3. When a sample of essential oil is taken the corresponding forms will be attached to it for transport to RDRL and analytical control.

4. Samples must be transported in containers of aluminium or preferably glass on which the material has no action. They shall be clean, dry, and free of all odours.

5. Precautions shall be taken to protect the samples, the material being sampled, the sampling implement and the containers for samples from contamination.

6. To draw representative samples, the content each container selected for sampling must be mixed thoroughly, by shaking, stirring, or both, by suitable means or by rolling.

7. The sample containers must be of such size that they are almost but not completely filled by the sample. The head space shall be between 5 or 10% of the volume of the container depending upon the method of transport adopted.

8. Each container must be sealed air-tight after filling and marked with identification particulars to allow comparison with corresponding forms.

9. Samples shall be stored in a cool place and protected from light and excecessive variation of temperature.

When the samples arrive at the central analytical laboratory they are subjected to GLC-analysis. A diagram is made showing the variation of the main constituents-yield with harvesting/distillation time. This being done over the period of one year will give a basis for determining the best harvesting time. The analytical conditions should be kept constant and the same for most essential oils to allow comparison between standard oils and samples. The analysis by RDRL and the reporting back to the herbal farms is a regular operation which will give the officers at the individual farms a much needed backup. It is of importance to develop a close connection between the farms and the central analytical laboratory. Especially, when the number of plants will increase in the future, access to reliable analytical results will allow the officers in the field to evaluate their own work.

To improve the results from the experimental herbal farms, emphasis should be given to plant selection and in this context more aid in the agricultural sector is needed. As this selection of plants is made for their essential oil quality, the reporting system established for the Cymbopogon oils must be continued on a regular basis with RDRL also for other plants.

D. Quality Assessment in Essential Oil Production

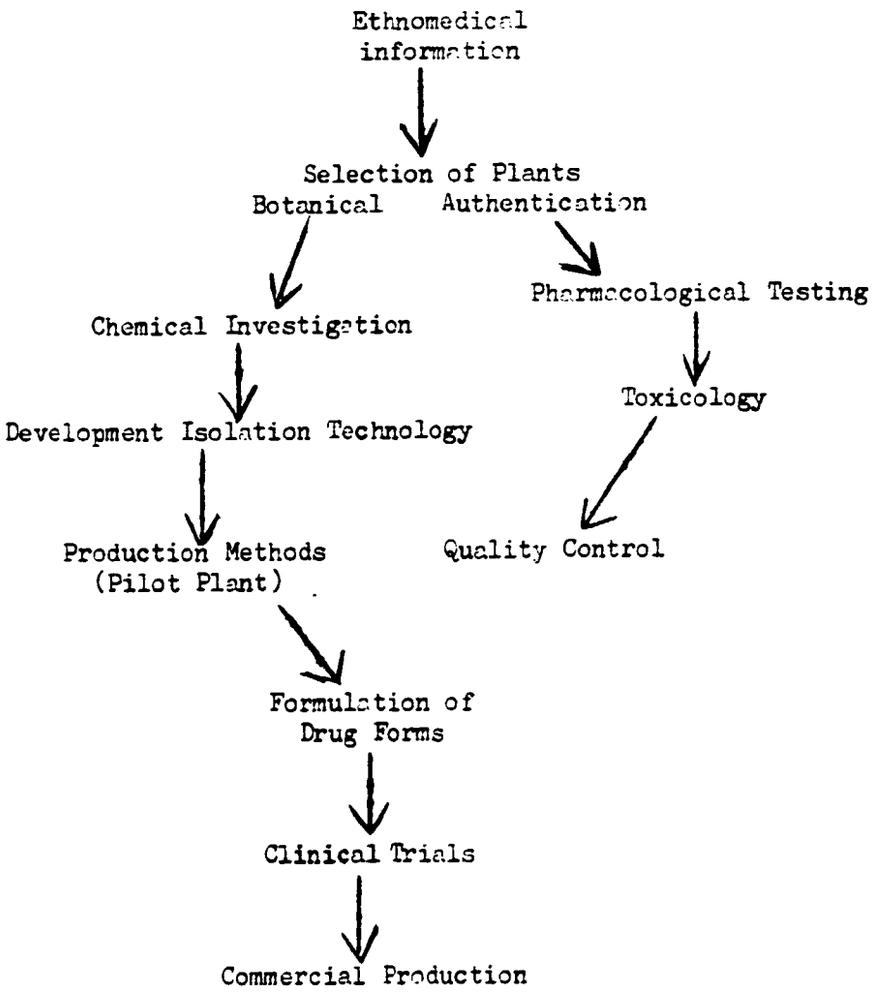
To ensure proper quality characteristics in the essential oils produced it is necessary that the aromatic plant material be standardized. Information on the proper identity of the basic plant material, harvesting season for optimum yield and relevant analytical data of interest to the consumers, producers and traders in essential oils must follow each batch of oil. For many essential oils produced on test scale in Nepal, the Indian Standards Institution* has already described standard elementary methods. These methods are, however, of little value for the introduction of essential oil on the international market. It is therefore of the utmost importance that the samples of essential oils commercially available be brought regularly to RDRL for analysis and comparison. The analytical method of choice for this ongoing investigation is gas liquid chromatography. The booklets issued by ISI also contain definitions of the usual terms met with when an essential oil is evaluated and RDRL should use the same defined terms when competing on this "local" market. For the basic investigation of an essential oil, samples from various places/sources must be collected during a whole season, the samples coded and compared so that a total picture of the quality of the product can be had.

For the quality assessment of essential oils produced for export, it is extremely important that the oils meet the requirements of international markets. It therefore involves the determination of certain characteristics and comparing them with defined standards, set down for each oil or product. In any process where a batch of raw materials is transformed into another product through one or more intermediate stages, the quality of the final product is assured through continuous control of the operations. The quality control steps of essential oils may follow the operation chart cited below:

Plant material	Botanical c., selection etc.
Distillation	Load, temperature, time
Crude oil control	Optical rotation, specific gravity, refractive index, ester value etc.
Final analytical control	GLC-analysis
Storage	GLC-analysis

In all the steps concerning storage, shipment and analysis of an essential oil the problem of inert and tight containers arises. It cannot be stressed enough the importance of selecting the appropriate container for the essential oils. The essential oils are volatile and often prone to chemical changes due to influence of metals, air, light, oxygen and temperature. The most common materials for essential oils are aluminium and glass. Glazed containers are also often used.

E. Screening of Medicinal Plants



At the moment this part of the ongoing research (screening of medicinal plants) belongs more to the pharmacological and microbiological section. The expert has given consideration to the future problems in this field by putting on order equipment for low pressure liquid chromatography. In the near future the phytochemical section will deliver purified extracts and pure compounds for pharmacological/microbiological testing.

The "flow chart" for the screening of medicinal plants is shown in the previous page. The "flow chart" clearly states the position of the different research sections in this type of investigations.

F. Training

1. Training General
2. Literature, books
3. Instrumentation maintenance
4. HPLC
5. Column chromatography
6. Spectroscopical identification
7. Seminars
8. Microcomputer
9. Back-up equipment for quality
10. Follow-up of essential oil analysis.

1. Training General

Training has been imparted by discussions, demonstrations and seminars in the laboratory. The expert wants to point out that a stay of one year is a very short time for proper training, but that the local staff should be able to get on when a working programme is left. The training has to a large extent been an encouragement of local staff to get experience while the expert is present in the laboratory and the stress has been put on the reliability of results obtained.

2. Literature, books

As a research laboratory, being "up-to-date" with scientific literature is a necessity. Nepal is badly situated in this respect. The delivery of scientific journals to the library of the RDRL is slow and irregular, a fact which causes problems. Moreover, obtaining copies of relevant publications is equally slow and irregular. The expert made a recommendation to the University of Oslo for a regular service and a model case was made for Dr. Timila Shrestha on literature search (computer search) and procurement of publications. In the experts opinion such a contect with an external source (university) would greatly improve the "library service".

To encourage the idea of self-education the UNIDO experts drew up a list of books in their respective areas (see CTA's report).

3. Instrumentation maintenance

In view of the upgrading of the instruments available in RDRL it becomes increasingly evident that some sort of regular maintenance is necessary. It is therefore most important that local staff be trained in instrument servicing. Installation of new equipment must be followed immediately with an introduction to the servicing of the instrument. It is also necessary that a regular maintenance programme be started and that the instruments be looked after at regular intervals.

4. HPLC

Equipment for HPLC-analysis has been installed and applied to the investigation of active constituents in Valeriana wallichii. By practical experiments the usefulness of HPLC equipment has been demonstrated. The Waters instrument is equipped with two detectors (Refractive index det. and a spectrophotometric det.) to allow the analysis of the "essential drugs" of Nepal. The instrument can easily be equipped with a gradient unit if this becomes necessary in the future. HPLC analyses of drugs are encouraged and model analyses will be undertaken during the next year to get practical experience. It is strongly recommended that model analyses be carried out often as the regular use of the HPLC instrument will give the local staff much needed training. This training can best be had through personal experience. Use of HPLC in quality control work will also require the use of organic solvents in larger quantities than usual. In future, planning the purchase of these solvents for model experiments will be most useful.

5. Column chromatography

One of the main problems of a research laboratory concerned with the isolation of active ingredients in medicinal plants is to have available suitable column chromatographic equipment. Low-dead volume columns, organic solvent pumps, and a fraction collector have been installed for this purpose. The advantage of this equipment has been demonstrated and the equipment applied to the isolation of single valepotriates from Valeriana wallichii.

6. Spectroscopical identification

For the identification of isolated compounds a modern analytical laboratory would have a mass spectrophotometer available. This instrument is considered too expensive for RDRL at the moment, but a Fourier Transform Infrared Spectrophotometer can be considered as a good second choice for this purpose. This FT-IR instrument is capable of giving a spectrum of a very small amount of material. In due course when a spectrum library has been established at RDRL this instrument will "fill the gap". At the moment this instrument has not yet been purchased and no training can be given.

7. Seminars

After an initial phase where the experts were asked to give lectures, regular weekly seminars have been arranged for the Quality Control Department. It is the opinion of the expert that these form an absolutely essential part of the work in a research laboratory. Judging from the experience gathered since July, when the seminars started, these weekly meetings are well received by the local staff. They also encourage the participation of the staff in scientific discussions and give them a possibility of some training in the giving of lectures.

8. Microcomputer

The concept of computerization has been embraced by members of the RDRL.

To encourage the understanding of the fundamentals of computer usage and the application to statistical calculation, leading to the filing of results, microcomputers, with necessary software were purchased. One instrument arrived in February, another in September. A small number of RDRL staff were sent on a training course to learn "BASIC" programming and these have taught other members of the staff the use of computers.

The experts (pharmacologist and analytical chemist) demonstrated the value of the available programmes. The pharmacology section were not able to exploit the calculation capability due to the shortage of data due in turn to the lack of animals. The chemical section of the laboratory

was quick to see the value of the filing programme and soon computer time became in great demand. The demand was so great that, after discussion with the senior members of the RDRL a second computer was ordered, this second machine - which arrived in September will be used primarily by the chemical section, and has, through a hard disk attachment and some additional hardware, the capability of handling a greater volume of data more rapidly.

9. Back-up Equipment for Quality Control

Judging by the number of analyses which will be undertaken by 'quality control' it is considered absolutely necessary that a new fluorimeter and an UV-spectrophotometer purchased. These instruments can and will be used also by the department of pharmacology.

10. Follow-up of Essential Oil Analyses

The expert has encouraged regular analysis of the essential oils produced by the experimental farms in Hetauda and Pokhara. The results of these analyses are again distributed to the farms to facilitate selection of plants.

At the same time laboratory scale distillation of plants containing essential oils are screened by means of GLC standard methods. The flora of Nepal has a rich spectrum of plants containing aromatic compounds and a regular screening of these will be continued.

G. Work Plan for Short to Medium Term at the RDRL

1. Essential Oils

a. Each year 20 plants containing essential oils should be screened for their potential as raw material for industrial essential oil production.

b. The plants passing the previous point should be investigated as to the seasonal variation of the constituents of the essential oil (estimation 5 plants/year).

c. The plants mentioned above should be grown in the herbal farms to investigate the possibilities of large-scale growing.

d. Points a,b and c will lead to a continuous programme for about 5 plants while a screening of 20 new plants is being carried out.

2. Medicinal Herbs

a. Routine pharmacological screening procedures for specific activities pointed out by the pharmacological experts should be established.

b. Plants passing point a must be investigated by chemical methods to establish nepalese standards. This does not necessarily mean that single compounds will be isolated but that plant material must be collected from all over Nepal and an average sample analyzed.

c. For Valeriana wallichii a long-term stability test procedure has started and will be continued for another year. This chemical analysis should be accompanied by a through pharmacological testing of the sedative effect of the standardized (analyzed) extract.

d. The screening test being carried out (point 'a') by the RDRL ought to lead to 2-4 plants a year which deserves closer investigation. The number of plants being screened could favourably amount to 20-30/year.

3. Quality Control

a. By means of the microcomputer all the analyses of drugs being done during the period of the last ten years should be put on a computer file for easy retrieval and comparison.

b. A programme for filing and updating a register of the medicinal plants of Nepal should be made and the plants put on the file.

c. The application of HPLC to the analysis of compounded drugs should be investigated by running 20-30 preparations as model problems.

Multi-purpose extractor

The 5 litre Multi-purpose Extractor combines the three types of extraction process commonly used in technical and industrial laboratories:

- Liquid/solid extraction
- Liquid/liquid extraction by upwards displacement
- Liquid/liquid extraction by downwards displacement

Using the minimum number of parts it allows rapid, easy conversion from one type to another and also permits recovery of solvent and extract after extraction. The unit features a 5 litre capacity extractor body working from a 20 litre wide-necked flask (Cat. No. 11EX). The glassware has been designed to allow complete adjustability of phase disengagement areas on conversion from one type to another and on changing the liquid system in a liquid/liquid extraction process. A demountable syphon allows conversion to liquid/solid extraction and a glass fibre thimble is provided. All conversions may be carried out without moving the flasks or extractor bodies from their initial settings. Rapid drainage is possible by means of Rotaflo stopcocks incorporated in the adapters used for inter-conversion. The extractor is easily assembled and dismantled for cleaning purposes and is supplied complete with frame and the necessary clamps, bosses and supports. The unit is compact, overall measurements being: 0.46 m x 0.91 m x 2.69 m height.

It can be supplied either with or without an 'Electrothermal' heating mantle, for 110V or 240V operation, and a control unit fitted with special mounting brackets.

Operation

1. Liquid/liquid extraction downwards displacement

The glassware is assembled as shown in illustration. Heavy phase solvent is poured into the extraction body followed by the solution to be extracted. The volume of either phase is then adjusted so that the bottom of the distributor EX 11/9 is completely immersed. During this operation heavy phase is forced up from the disengagement area and through the annulus between the detachable sleeve EX 11/10 and the take off tube EX 11/11; the latter is then adjusted so that heavy phase returns to the flask. Refluxing solvent enters the solution to be extracted via the horseshoe-shaped distributor which is perforated on the underside.

2. Liquid/liquid extraction upwards displacement

Assembly of the glassware is the same as for downwards displacement except that the sleeve EX 11/10 is removed and distributor EX 11/8 replaces distributor EX 11/9. The solution to be extracted is poured into the extractor body and the height of the take-off tube is adjusted so that it is just above the liquid level. Refluxing solvent enters the solution to be extracted via the horseshoe-shaped distributor which has perforations on the upper side.

3. Liquid/solid extraction

Assembly of the unit for Soxhlet extraction is shown in illustration B. The sample to be extracted is placed in the glass fibre thimble or, alternatively, supported directly in the extractor body. Refluxing solvent from the condenser is directed on to the sample and returned to the flask by a syphoning action.

4. Solvent recovery

Assembly C permits recovery of solvent and extract after extraction without the necessity to dismantle or empty the apparatus. The plug EX 11/17 is fitted and the condenser C 23/77/3 arranged in the distillation position using the recovery bend SH 2/77.

Instructions covering assembly and operation are supplied with each unit.

ANNEX - III

Caesalpinia decapetala (Roth) Alst.

The protestations of the experts in relation to record keeping, correct naming of plants and the collection of voucher specimens have not been universally accepted as significant.

The species Caesalpinia sepiaria is mentioned in 'Medicinal plants of Nepal' and Caesalpinia bonduc is mentioned in the plant list in appendix '12' in the Technical Report: Pharmacology Laboratory by Dr. J.P.G. Williams. The description of the distribution given in the Flora of British India suggests the species occuring in the Himalaya was C. bonducella. When reviewing the description of these species the expert came across a passage which underlines the difficulties of plant identification and the need for voucher specimens with every collection.

ANNEX - IV

List of "Essential Drugs" for Nepal

Anaesthetics

General

Ether

Nitrous oxide

Inj. Diazepam

Halothane

Thiapental sodium

Local

Lignocaine

Analgesics, antipyretics, non-steroidal anti-inflammatory drugs and drugs to treat gout

Acetylsalicyclic acid

Ibuprofen

Indomethacine

Oxyphenbutazone

Paracetamol

Allopurinol

Colchicine

Probenecid

Miscellaneous

Magnessium sulphate paste, Inj., Emetine for scorpion bite

Eusol

Rectified spirit (Ethyl alcohol)

Analgesics, Narcotic and Norcotic antagonists

Morphine

Pethidine — Dangerous drugs only to be prescribed by Doctors

Nalorphine

Pentazocine

Anti-Allergics

Anti-Histaminics

Chlorpheniramine

Promethazine

Antidotes, chelating agents etc.

Atropine

Activated charcoal

Pralidoxime

Dimercaprol

Anti-epileptics

Diazepam Inj.

Phenobarbitone

Carbamazepine

Ethosuximide

Phenytoin

Anti-infective Drugs

Anthelmintics

Mebendazole

Bephenium

Piperazine

Niclosamide

Antibacterial Drugs

Ampicillin

Benzyl Penicillin

Cloxacillin

Gentamycine

Sulphadimidine

Benzathine Benzyl Penicillin

Chloramphenicol

Erythromycin

Phenoxymethyl Penicillin

Sulphamethoxazole + Trimethoprim

Pyrizinamide

Thiacetazone + Isoniazide + PAS

Systemic Antifungal Drugs

Griseofulvin
Amphotericin B

Antimigrane Drugs

Ergotamine

Antineoplastic Drugs

Ergotamine
Busulphan
Vancristine
Chlorambucil
Azothiaporine
Cyclophosphamide
Methotrexate
Daxunorobicin
Vinblastin

Antiparkinsonism Drugs

Levodopa
Total Belladona Alkaloid
Trihexyphenidyl
Diphenhydramine

Blood-Drugs Affecting Anti-anaemia

Cyanocobalamine or Cobalamine Inj.
Ferrous salts
Folic acid
Iron Inj.

Anticoagulants and Antagonists

Heparin
Warfarin
Phytomenadione
Tetracycline
Nitrofurantoin

Procaine benzyl Penicillin
Metronidazole (for Anaerobas)
Doxycycline

Antifilarial Drugs — Diethyl Carbamazine

Anti-leprotics
Dapsone
Rifampicin
Clofazamine

Amoebicides

Metronidazole
Emetine
Diiodohydroxyquine
Diloxanide
Iodochlorhydroxyquine

Anti-malarials

Chloroquine
Primaquine
Sulphamethopyrazine + Pyrimethamine
Amodiaquine
Pyrimethamine

Anti-Kalazar

Stibogluconate

Anti-Tuberculosis Drugs

Isoniazid
Ethambutol
Rifampicin
Ethionamide
Streptomycin

Plasma Substitute

Dextran 70
Dextran 40

Cardiovascular Drugs

Antianginal Drugs

Glyceryl Trinitrate

Propranolol

Isosorbide dinitrate

Antiarrythmic

Lignocaine

Propranolol

Verapamil Inj.

Procainamide

Quinidine

Antihypertensive

Hydrolazine Inj.

Propranolol Inj.

Reserpine

Hydrochlorothiazide

Methyldopa

Cardiac Glycosides

Digoxine Oral & Inj.

Drugs used in shock and Anaphylaxis

Isoprenaline Inj.

Epinephrine

Dermatological Drugs: Topical anti-infective

Tr. Iodine

Nitrofurarone oint.

Acriflavin

Mercurochrome

Neomycin

Tr. Benzoin comp.

Anti-inflammatory

Betamethasone esters
Liniment Methylsalicylate
Hydrocortisone
Counter irritant

Astringent

Aluminium acetate
Calamine lotion
Zinc paste and cream

Fungicides

Benzoic acid + Salicylic Acid
Mystatin
Gentian-violet

Keratoplastic

Salicylic acid
Coal Tar

Scabicidal and pediculocide

Gamma benzene Hexachloride
Benzyl Benzoate

Diagnostic agents

Tuberculin P.P.O.
Fluorescine

Radio-Diagnostic agents

Meglumine
Adipiodine Meglumine
Iopanoic acid
Sodium amidotriazoate
Barium Sulphate

Diuretics

Furosemide
Triameterine
Mannitol I.V.

Hydrochlorothiazide

Spironolactone

Gastrointestinal Drugs

Antacids

Aluminium Hydroxide

Calcium Carbonate

Magnessium hydroxide

Antispasmodic

Atropine

Belladone Dry Extract

Anti-haemorrhoidals

Xylocain ointment

Adstringent and anti-inflammatory-preparation

Cathartics

Senna

Castor oil

Mag. Sulphate

Bisacodyl

Liquid Paraffin

Anti-Diarrhoeal

Atropine + Diphenoxylate Hol.

R.D. Sol Replacement SD

Metronidazole (Ciardiasis Amoebiasis)

Tetracycline (Bacterial Diarrhoea)

Replacement Solution

R.D. Sol

Drugs for Flatulence

Dimethylpolysiloxane
Volatile oils
Sodium Bicarbonate
Activated charcoal

Hormones and Synthetic Substitutes

Dexamethasone
Prednisolone
Hydrocortisone

Androgens

Testosterone esters

Estrogens

Ethinyl Estradiol

Oral Contraceptives

Norethisterone
Ethinylestradiol

Progestogens

Norethisterone

Thyroid and Antithyroid Drugs

L-Thyroxine Sodium
Carbimazole
Lugol's Iodine

Insuline and Oral Anti-Diabetics

Compound Insulin
Tolbutamide
Crystalline Insulin
Chlorpropamide

Immunologicals

Anti-rabies serum

Tetanus Antitoxoid

Diphtheria Antitoxin

Anti-snake-venum

Vaccines

B.C.G.

Measles

Rabies

T.A.B.

Yellow fever only to international travellers from Yellow fever areas

Dephtheria-Pertussis-tetanus

Polio

Tetanus

Muscle relaxants peripherally acting and antagonist

Gallamine

Neostigmine

Suxamethonium

Eye + ENT Preparations : Tropical, Anti-infection

Silver Nitrate

Chlortetracycline

Chloramphenicol

Gentamycin

Polymyxin-B Sulphate + Neomycin + Bacitracin + Dexamethasone

Sulphacetamide

Betamethasone sod. phos.

Dexamethazone

Oxymetozoline

Local Anaesthetic

Xylocaine

Miotics

Pilocarpine

Eserine

Mydriatic

Atropine

Epinephrine

Homatropine

Systemic

Acetazolamide

Oxytocics

Ergometrine

Oxytocin

Psychotherapeutic Agents

Amitryptiline

Chlorpromazine

Haloperidol

Inj. Trifluopromazine

Chlordiazepoxide

Diazepam

Imipramine

Prochlorperazine

Respiratory tract Drugs

Aminophylline

Epinephrine

Salbutamol

Ephedrine

Codeine

Solution Correcting water Electrolytes, acid base disturbances

Oral rehydration salt

Parenteral

Glucose 5%

Haemacel

Ringer's Solution

Sodium Bicarbonate 2%

Glucose 50%

Pot. Chloride Inj.

Sodium Chloride (0.9%)

Water for inj.

Vitamins and minerals

Ergocalciferol

Cal. Gluconate Inj.

Pyridoxine

Riboflavine

Yeast

Ascorbic acid

Nicotinamide

Retinol

Thiamine HCl.

ANNEX - V

Job Description

(DP/NEP/80/003/11-04/32.1.D)

Post title :	Chemist (Analytical)
Duration :	12 months
Date required:	ASAP
Duty station :	Kathmandu/Nepal
Purpose of project :	To develop further the existing facility for the production of plant-derived pharmaceuticals at the Royal Drugs Research Laboratory, Kathmandu/Nepal.
Duties :	The expert will be expected to conduct quality assessment on pharmaceutical products. Specifically he will develop analytical methods for determining the content of active ingredients in medicinal and aromatic plants and all products prepared from these plants. The expert will also be expected to prepare a final report, setting out the findings of his mission and his recommendations to the Government on further action which might be taken.
Qualifications :	Ph.D. in Organic or Analytical Chemistry. A thorough working knowledge on the use of various analytical instruments such as IR, UV, NMR, GLC, HPLC etc. is essential and experience in quality control of pharmaceutical products.
Language :	English.

Background information

Nepal is a country with a population of approximately 12 million with an annual growth rate of 2.4 per cent. Over 90 per cent of the people live in rural areas and over 60 per cent of them in the mountain zones. Most of the rural folk utilise plant-preparations for their therapeutic requirements and the traditional system of medicine is very similar and derived from the Ayurvedic system prevalent in the Indian Sub-Continent. The wealth medicinal plants can be considered as one of the important natural resources of Nepal. The Kingdom of Nepal lies in the Central Sector of the great Himalayas and occupies a third of the total length. The diversity of physiography due to attitudinal and climatic variations has brought about a great variety of species of plants within the flora of this small (Area: 145,305 sq km) country. Much of this flora is used in medicine and the Royal Drugs Research Laboratory is responsible for the R and D efforts leading to the production of pharmaceuticals based on the traditional remedies. Currently the RDRL

produces essential oils and extracts of plant material for pharmaceutical purposes albeit on a small scale, and conducts research on several aspects of the flora.

In 1972 part of the production unit was converted to a manufacturing corporation under the name Royal Drugs Ltd. (R.D.Ltd.) and the RDRL remains the research and development agency.

The present project seeks to strengthen the existing facilities of RDRL, to include pilot scale facilities, for research and development of plant-derived pharmaceuticals.

ANNEX - VI

Terpene Standard Present at RDRL as at January 1983

Name	Origin
ALPHA-PINENE	RDRL
ALPHA-TERPINENE	RDRL
ALPHA-TERPINEOL	RDRL
ANETHOL	RDRL
BORNEOL	RDRL
BORNYLACETATE	RDRL
CAMPHENE	RDRL
CARVANOL	RDRL
CARVON	RDRL
CARYOPHYLLENE	RDRL
CITRAL	RDRL
DELTA-3-CARENE	RDRL
EUGENOL	RDRL
FARNESOL	RDRL
GAMMA-TERPINENE	RDRL
ISOBORNEOL	RDRL
ISOEUGENOL	RDRL
LIMONEN	RDRL
LINALOOL	RDRL
LINALYLACETAT	RDRL
MENTHOFURAN	RDRL
MENTHON	RDRL
METHOXYCARVANOL	RDRL
NONANAL	RDRL
PARA-CYMOL	RDRL
PIPERITON	RDRL
PULEGON	RDRL
THYMOL	RDRL

ANNEX - VII

Terpene Standards Procured

Name	Origin
AMYLACETATE	ROBERTET
AMYLCINNAMIC ALDEHYDE	ROBERTET
AMYRIS (SANDALWOOD W.I.)	ROBERTET
BENZALDEHYDE	ROBERTET
BENZYLACETATE	ROBERTET
BENZYLALCOHOL	ROBERTET
BERGAMOTE OIL	ROBERTET
CISTE OIL	ROBERTET
CITRAL	ROBERTET
CITRONELLA JAVA OIL	ROBERTET
CITRONELLA SRI LANKA OIL	ROBERTET
CITRONELLA TAIWAN OIL	ROBERTET
CITRONELLAL	ROBERTET
CITRONELLOL	ROBERTET
DIETHYLPHTALATE	ROBERTET
GERANIOL	ROBERTET
GERANIUM ALGERIA OIL	ROBERTET
GERANIUM BOURBON OIL	ROBERTET
GERANYLACETATE	ROBERTET
HYDROXYCITRONELLAL	ROBERTET
IONONE	ROBERTET
LAVANDIN ABRYALIS OIL	ROBERTET
LAVANDIN SUPER OIL	ROBERTET
LEMONGRASS INDIAN OIL	ROBERTET
LINALOOL	ROBERTET
LINALYLACETATE	ROBERTET
METHYLIONONE	ROBERTET
NEROL	ROBERTET
NEROLI OIL	ROBERTET
PALMAROSA OIL	ROBERTET
RHODINOL	ROBERTET

Name	Origin
RHODINYL ACETATE	ROBERTET
SANDALWOOD INDIAN OIL	ROBERTET
VETIVER INDIAN OIL	ROBERTET
VETIVERYLACETATE	ROBERTET

ANNEX - VIII

Batch Control of Palmarosa Oil Distillation

Vrindaven Herbal Farm, Hetauda

(June/July 1983)

Batch No.	Distill. Date	Yield	Peak 1	Peak 2
1	8/7	0.27	11.77	81.06
2	8/7	0.28	15.99	76.73
3	8/7	0.29	13.94	78.77
4	9/7	0.25	14.81	80.23
5	9/7	0.27	11.59	82.6
6	9/7	0.25	12.98	80.83
7	10/7	0.26	13.51	80.66
8	10/7	0.23	13.1	79.49
9	11/7	0.28	12.97	81.22
10	11/7	0.30	11.22	80.44
11	11/7	0.31	14.04	24.64
12	12/7	0.30	13.8	79.1
13	12/7	0.25	13.9	79.5
14	13/7	0.24	13.2	80.6
15	13/7	0.31	14.9	78.9
16	13/7	[illegible]	13.2	78.1
17	14/7	0.29	13.5	79.5
18	14/7	0.27	13.1	80.5
19	5/7	0.27	13.4	80.8
20	5/7	0.26	13.5	78.9
21	6/7	0.24	21.6	69.7
22	8/7	0.32	13.8	77.6
23	7/7	0.28	19.1	75.7
24	7/7	0.16	11.3	83.1
42	10/6	0.19	23.15	69.33
43	11/6	0.20	22.71	70.72
44	11/6	0.20	24.89	68.22
45	12/6	0.20	22.76	68.94
46	12/6	0.20	19.58	74.77
47	12/6	0.20	22.45	71.46
48	12/6	0.20	21.43	72.53

ANNEX - IX

Batch Control of Citronella "Java" Oil Distillation
Vrindavan Herbal Farm, Hetauda
(May - August 1983)

Batch No.	Distill. Date	Yield	Peak 1	Peak 2	Peak 3
1	2/6	1.3%	44.35	17.14	17.32
2	2/6	1.4%	30.3	12.29	22.03
3	3/6	1.3%	45.3	13.58	17.04
4	31/5	1.3%	36.56	12.45	20.35
5	1/6	1.4%	26.64	9.37	27.92
19	26/7	0.96%	40.79	15.13	22.95
20	27/7	1.1%	48.27	14.14	21.2
21	27/7	0.9%	26.51	16.8	30.82
22	27/7	0.9%	22.19	19.58	26.37
23	27/7	1.3%	28.29	18.53	27.09
24	28/7	1.7%	42.52	16.54	21.02
25	28/7	0.9%	45.29	15.06	21.37
26	29/7	0.9%	32.39	20.06	22.44
27	29/7	1.0%	48.6	14.63	22.09
28	29/7	0.9%	23.21	20.09	24.78
29	29/7	0.9%	21.51	19.51	25.55
30	29/7	1.0%	24.74	18.71	24.08
31	31/7	0.9%	37.95	17.2	22.4
32	1/8	0.9%	32.71	15.24	29.37
33	1/8	0.9%	42.96	15.43	20.59
34	1/8	1.1%	19.66	14.65	34.82
35	2/8	1.0%	23.14	18.31	32.59
36	2/8	0.7%	26.14	17.33	37.91
37	3/8	0.8%	27.49	15.56	38.81
38	3/8	0.8%	37.2	17.39	23.21
39	3/8	0.9%	27.8	19.81	33.55
40	5/8	0.9%	37.96	16.59	20.86
41	20/8	1.1%	42.54	16.04	22.82
42	7/8	1.0%	32.66	16.47	29.0
43	7/8	1.0%			

Batch No.	Distill. Date	Yield	Peak 1	Peak 2	Peak 3
44	7/8	1.3%	39.16	15.37	22.0
45	8/8	1.3%			
47	8/8	1.3%	92.78	14.39	25.15
48	8/8	1.3%	37.96	16.11	23.06
49	9/8	0.9%	37.45	15.74	26.43
50	9/8	0.3%	43.61	14.18	22.81
53	10/8	1.0%	34.5	15.31	25.04
54	10/8	1.2%	36.54	14.96	26.34
51	9/8	1.1%			
52	10/8	1.1%			

ANNEX - X

Batch Control of Lemongrass "Nagaland" Oil Distillation
Vrindavan Herbal Farm, Hetauda
(June 1983)

Batch No.	Distillation date	Yield essential oils	Main Constituents Peak 3	Peak 4	Total
6	4/6	0.31%	28.4%	38.7%	67.1%
7	4/6	0.26%	28.3%	38.3%	66.6%
8	4/6	0.34%			
9	5/6	0.41%	30.0%	44.3%	74.3%
10	5/6	0.34%	25.4%	35.4%	70.8%
11	6/6	0.34%	31.3%	41.3%	72.6%
12	6/6	0.36%	26.5%	34.5%	61.0%
13	6/6	0.41%	30.0%	39.9%	69.9%
14	6/6	0.41%	29.4%	39.3%	68.7%
15	7/6	0.39%	29.9%	40.5%	70.4%
16	7/6	0.32%	30.2%	39.9%	70.1%
17	8/6	0.41%	31.4%	41.6%	73.0%
18	8/6	0.38%	26.6%	35.9%	62.5%
19	8/6	0.38%	31.1%	41.05%	72.1%
20	13/6	0.31%	27.3%	37.9%	65.2%
21	13/6	0.30%	23.5%	35.7%	59.2%
22	13/6	0.35%	29.7%	39.8%	69.5%
23	16/6	0.32%	30.0%	41.8%	71.8%
24	16/6	0.32%	-	-	-
25	17/6	0.34%	29.2%	39.9%	69.1%
26	17/6	0.33%	32.2%	45.8%	78.0%
27	17/6	0.34%	29.2%	42.2%	71.4%
28	18/6	0.34%	32.5%	44.9%	77.4%
29	16/6	0.38%	31.04%	49.	80.7%
30	19/6	0.20%	30.6%	43.2%	73.8%
31	19/6	0.29%	26.5%	36.1%	62.6%
32	19/6	0.27%	29.8%	41.4%	71.2%
33	20/6	0.27%	30.7%	42.7%	73.4%
34	20/6	0.33%	31.6%	44.0%	75.6%

Batch No.	Distillation date	Yield essential oils	Main Constituents Peak 3	Peak 4	Total
35	20/6	0.31%	18.7%	28.9%	47.6%
36	20/6	0.29%			
37	21/6	0.34%	32.2%	43.6%	75.3%
38	21/6	0.28%	24.5%	34.0%	58.5%
39	22/6	0.22%	26.9%	38.1%	65.0%
40	22/6	0.35%			
41	22/6	0.30%			

Peaks 3 and 4 are those of Citral a and Citral b.

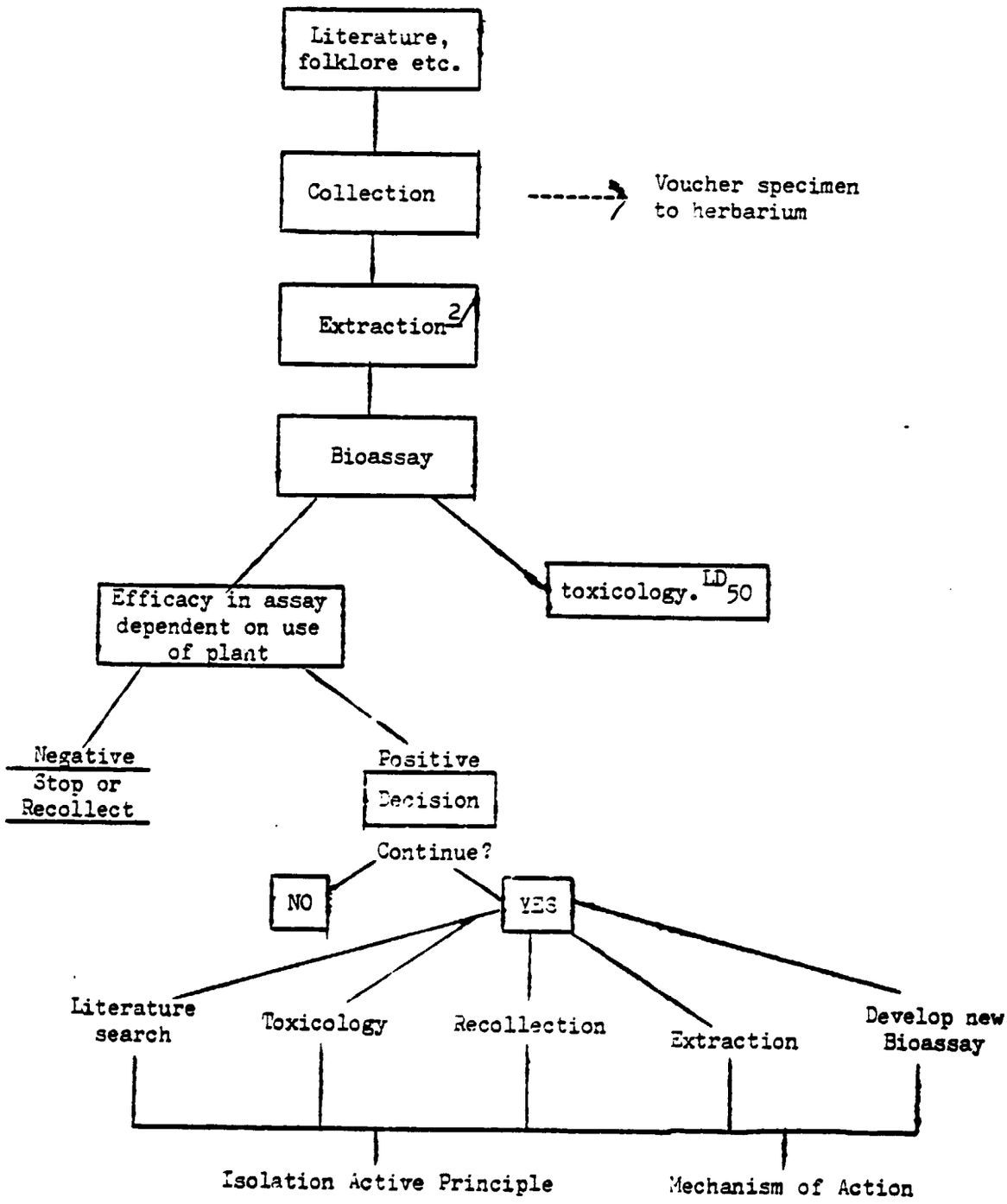
ANNEX - XI

Harvesting/Distillation Time for Cympogan Oils at Hetauda and Tarhara Herbal Farms

Plants	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Cymbopogon martini var. motia (Palmarosa)					xxxx	xxxx	xxxx	xxxx	xxx			
Cymbopogon flexuosus (lemongrass)				xxxx	xxxx	xxxx	xxxx	xxxx	xxx			
Cymbopogon winterianus (Citronella)				xxxx	xxxx	xxxx	xxxx	xxxx	xxx			

ANNEX - XVII

Scheme for Screening of Medicinal Plants 1/



1/ See also "Traditional Pharmacopoeias Revisited. UNIDO/10.511 25 Aug. 1982.
2/ May not be needed, reports may show that the plant is eaten.

ANNEX - XVIII

Fellowship Programme Suggested by Experts

It is our opinion that the fellowship programme should be related to the aims of the Royal Drugs Research Laboratory and to enhance its research capability. We recommend that staff be sent for M.Sc. or Ph.D. training and that this training be directly related to the projects of the Royal Drugs Research Laboratory as conceived in its long term planning. We see little benefit for RDRL (or Nepal) when a M.Sc. or a Ph.D. candidate on return to Kathmandu is faced with a situation where her/his experience and training cannot be utilized due to lack of facilities and relevant projects. Before the candidates leave there should be a clear understanding by the candidate, the supervisor at RDRL and the host university on the relationship of the training to the research activity of RDRL. It does not strengthen RDRL just to send people away for training without further specification. We We believe that the most appropriate type of training of members of RDRL would be that which combines industrial experience with academic education (e.g. CASE awards and sandwich courses in UK).

Assuming that RDRL wishes to do research in the fields of medicinal plants/drug production we would recommend the following areas of training:

1. Chemist/Pharmacist - M.Sc.-Ph.D.

   Instrumental analysis related to the stability testing of drugs with emphasis on chromatographic methods.

2. Pharmacist - M.Sc.-Ph.D.

   Formulation of drugs and pharmacokinetics. The training must be linked to a pharmaceutical company.

3. Pharmacist - M.Sc.

   Formulation of drugs and pharmacokinetics. The training must be linked to a pharmaceutical company.

4. Chemist/Pharmacist/Biologist - M.Sc.-Ph.D.

   Toxicology (general).

5. Chemist/Pharmacist/Biologist - M.Sc.

   Toxicology (specializing in techniques).

6. Pharmacology - M.Sc.-Ph.D.

7. Research and Development Management in Pharmaceutical Industry - MIT-Courses, Amsterdam.

8. Microbiologist - M.Sc.-Ph.D.

   Quality assessment of pharmaceutical products. The training should be linked to a Governmental Control Institute and a pharmaceutical company, preferably in the USA.

9. Basic computer training course for at least one pilot plant operator.

10. An on-the-job training for approx. 6 months in an Indian firm working in the phytochemical sector for the technicians at the pilot plant (at present there are three).

11. An on-the-job training at WALSONS (possibly 3 months) for the boiler mechanic.

ANNEX - XIX

Recommendations for Primary Record Keeping in the Examination of Plant Materials for Biological Activity

It is assumed that a research group consisting 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 programme 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 (i.e. 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 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 chemist to offer assistance and advice in questions concerning quality control.

P1

Plant Collection Form to be filled in by Field Collector
as far as possible IN THE FIELD

1. Plant name: _____ Nepali 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 note book ref:

13. Plant collected by: _____ Signature _____

14. Plant delivered to: _____ Signature _____

15. Date of delivery: _____

Copies to:

Project Director
Chemist
Pharmacologist
Microbiologist

P2

Chemical Extraction Form

1. Plant name: _____ Nepali name: _____

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

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

6. Specimen No.: C/SSS/CC/DD,DD,DD/EE

7. Date received: _____

8. Condition of material on date of extraction: _____

9. Drying method: _____ 10. Weight Dried Plant _____

11. Date extraction commenced: _____ 12. Date extraction completed _____

13. Solvent: _____ 14. Time: _____ 15. Temperature _____

16. Deionized Y or N 17. Concentration procedure: _____

18. Residual weight: _____ 19. Dry weight equivalent: _____

20. Aqueous solubility: _____ 21. Appearance: _____

22. Amount sent for bioassay: _____ 23. Date sent for bioassay _____

24. Amount sent for microbiological testing: _____

25. Date sent for microbiological testing: _____

26. Comments:

27. Note book no. page no.: _____

28. Extraction carried out by: _____ 29. Signature: _____

30. Extract delivered to: _____ 31. Signature: _____

32. Date: _____

Copies to:

Project Director
Botanist
Pharmacologist
Microbiologist

P3

Bioassay 1.

Pharmacological testing

1. Plant name: _____ Nepali 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 _____ 16. ED_{50} _____

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

18. Note book no. page no. _____

19. Carried out by: _____ Signature _____

20. Date _____

21. Comment and recommendation by Senior Pharmacologist.

Copies to:

Project Director
Botanist
Chemist
Microbiologist

P4

Bioassay 2

Microbiological testing

1. Plant name: _____ Nepali 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 _____

14. Active: Y, N, E _____

15. Comments:

16. Note book 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 no., this should be allocated from the master list of plants SSS
4. Collection no. this is allocated by the botanist in the field and refers to his collection, this should also be recorded in the field note book.
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 eg. T, Thapathali, G, Godavari (Not yet fixed).
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 - 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 Note Book reference, page no.
13. } Name, printed and signature of collector.
14. }
15. } Name, printed and signature of person taking over the plant
16. } from 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 No. C indicates chemistry, SSS/CC/DD,DD,DD as P1. EE extraction No. 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 estimation of the Dry Weight Equivalent (19).

11-12 Dates extraction commenced and completed.

13-15 Conditions of extraction

16. Has the extract been deionised? 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
eg. 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. Appearence

22. Amount sent for biossa

23. Date sent

24. Amount sent for microbiological testing

25. Date sent

26. Comments

27. Note book no. and page no.

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 No. B=Bioassay, SSS,CC,D,D,D,D,DD as P1,2.
EE extract number as P2, TT bioassay number.

7. Date received from chemist

8. Dry weight equivalent from chemist's form.

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

10. Activity reported in literature. e.g. anthelminthic.

11. Appearence 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. Note book ref. no. page no.

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 No. M=Microbiological test SSS/CC/DD,DD,DD, as P1,P2,P3.
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 sol. 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 e.g. antibacterial - E.coli

13. Date test commenced

14. Active, Yes, No, Equivocal.

15. Comments

16. Note book ref. no. page no.

17. Name signature of person who carried out test and date.

18. Comment and recommendation by senior microbiologist.

Bioassay of Plant Material

The bioassay results may be negative, positive or equivocal.

If the results are equivocal then a higher dose range should be used if possible - or a different route of administration or a different 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 plants' activity and a higher dose range may be tried.

If the results are positive then no further action need be taken, it would however be valuable to know if the activity was always the same.

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 'Nepali' 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.

ANNEX - XX

Batch Control Form for the Production Units*

From: Batch No.

Name of the plant/type/selection:

Location and date of collection:

Location and date of distillation:

Amount of plant material collected:

Period of distillation:

Yield:

Storage conditions:

Comments: Date and Signature:

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* This form concerns the production of essential oils.

ANNEX - XXI

Purity Requirements for Gases for GC-analysis

Impurity levels (ppm) maximum	Nitrogen	Hydrogen
O_2	4	4
Moisture	4	4
CO_2	0.5	0.5
CO	0.5	1
N-Oxides	0.5	0.5
Ar	10	0
H_2	1	
N_2		100

ANNEX - XXII

List of Plants Growing in Nepal to be tested for the Content of Essential Oil and Evaluated as Raw Material for Future Production of Aroma compounds

S.No.	Family	Total No. of species	Species reported to contain essential oil/ (Common/Vernacular Name)	Distribution (Altitudes in Meters)	Availability
1.	Malvaceae				
2.	Pinaceae	2	i. Abies pindrow ii.A. spectabilis (Himalayan Silver Fir)	W(1800-2600) WCE(2500-3900)	F A
3.	Mimosaceae	5	Acacia farnesiana	WC(100-1520)	R
4.	Araliaceae	2	-	-	-
5.	Compositae	1	-	-	-
6.	Araceae	1	Acorus calamus (Bojho)	WC(1300-2550)	F
7.	Acanthaceae	1	Adhatoda vasica (Asuro)	WCE(180-2130)	F
8.	Rubiaceae	1	Adina cordifolia (Karma)	WCE (150-770)	A
9.	Rutaceae	1	Aegle marmelos (Bael)	WCE (200-1070)	A
10.	Compositae	1	Ageratum conzoides	WCE (180-2280)	A
11.	Labiatae				
12.	Mimosaceae	4	Albizzia labbeck	WCE (270-850)	F
13.	Liliaceae	9	i. Allium cepa (Pyaz) ii. A. sativum iii. A. wallichii	Cultivated in tropical and subtropical zone WCE (2130-4000)	 A
14.	Zingiberaceae	1	Amomum subulatum (Cardamom, Alaichi)	Cultivated in subtripical region of Eastern Nepal	A
15.	Compositae	10	i. Anaphalis contorta	WCE (450-4420)	A
16.	Ranunculaceae	10	i. Anemone elongata ii. A. rivularis iii. A. citifolia	WCE(1460-3350) CE (2000-4000) WCE(1300-2800)	F A F
17.	Labiatae	1	Anisomeles indica (A. ovata)	CE (200-2130)	F
18.	Annonaceae	1	Annona squamosa (Sarifa)	WE (180-1200)	F
19.	Umbelliferae	1	Archangelica officianalis	C (2440-2740)	F
20.	Sapotaceae	2	Bassia butyracea	CE (780-1430)	A
21.	Cassalpinaceae	5	Bauhinia variegata (Koiralo)	WCE (610-1830)	F

S.No.	Family	Total No. of species	Species reported to contain essential oil/ (Common/Vernacular Name)	Distribution (Altitudes in Meters)	Availability
22.	Boenninagausenia (Rutaceae)	1	Boenningausenia albiflora	WCE (400-3250)	A
23.	Umbelliferae	5	Bupleurum candollei	WCE (2080-3810)	F
24.	Papilionaceae	1	Cajanus cajan (Rahar)	Cultivated through out tropical region	A
25.	Verbenaceae	3	Callicarpa macrophylla	WCE (230-2130)	F
26.	Cannabinaceae	1	Cannabis sativa (Bhang)	WCE (180-2800)	A
27.	Cruc ferae	1	Capsella bursa-pastoris (Lady's purse)	WCE (1300-4660)	F
28.	Solanaceae	2	Capsicum annuum	Cultivated in tropical & subtropical region.	A
29.	Caesalpinaceae	6	Cassia fistula (Rajbriksha)	CE (260-1370)	F
30.	Pinaceae	1	Cedrus deodata (Deodar)	W (2100-2500)	F
31.	Umbelliferae	2	Chaerophyllom villosum	C (1520-2900)	F
32.	Gramineae	3	Chryspogon aciculatus (Andropogon aciculatus)	WCE (120-1600)	A
33.	Lauraceae	3	i. Cinnamoum camphora	C (1300-2130) & cultivated	F
			ii. C. tamala	WCE (500-1830)	A
34.	Menispermaceae	1	Cissampelos pareira	WCE (180-2960)	F
35.	Rutacae	3	i. Citrus aurantium	Cultivated in sub-tropical region	
			ii. C. medica		
			i. C. buchananiana	WCE (900-3750)	F
			ii. C. montana	WCE (1500-3960)	F
36.	Rubiaceae	1	Coffea sp.		
37.	Umbelliferae	1	Coriandrum sativum (Dhaniya)	Cultivated in tropical and subtropical region	A
38.	Cuminum (Umbelliferae)	1	Cuminum cyminum (Jeera)	Cultivated in tropical region	F
39.	Cupressus (Cupressaceae) (Graminae)	16	i. Cupressus torulosa flexuosus	WC (2400-2900)	A
			ii. C. pendulus (Andropogon nardus)	CE (180-2000)	F
			iii. C. stracheyi	C (200-1800)	F
40.	Cyperus (Cyperaceae)	11	i. Cyperus niveus	WC (100-2900)	F
			ii. C. rotundus (Mothe)	WCE (100-1600)	F
41.	Dactylis (Gramineae)	1	Dactylis glomerata	WC (1430-3500)	F

S.No.	Family	Total No. of species	Species reported to contain essential oil/ (Common/Vernacular Name)	Distribution (Altitudes in Meters)	Availability
42.	Papilionaceae	6	i. Dalbergia sericea ii. D. sisso	WC (150-1680) WCE (180-1220)	F A
43.	Thymelaeaceae	3	-	-	
44.	Labiatae	8	i. Elsholtzia flara ii. E. fruticosa (E. polystachya)	CE (1500-2590) WCE (1680-3500)	F F
45.	Umbelliferae	1	Foeniculum vulgare (Saunp)	Cultivated in sub-tropical and region	A
46.	Ericaceae	7	i. Gaultheria fragrantissima (Dhasingare) ii. G. trichophylla	CE (610-2900) CE (2700-4500)	A F
47.	Geraniaceae	13	-	-	-
48.	Zingiberaceae		H. spicatum	CE (260-2770)	F
49.	Sauruaceae	1	Houttuynia cordata (Gande)	WCE(1220-2440)	F
50.	Iridaceae	4	i. Iris decora ii. (I. nepalensis) (Padam Puskar)	C (1830-4850)	F
51.	Juiglandaceae	1	Juglans regia (Wallnut, Okhar)	WCE (1370-3000)	A
52.	Varbenaceae	1	Lantana camera	Weed in subtropical region CE	A
53.	Labiatae	5	i. Leucas cephalates ii. L. mollissima	WEC (180-2000) WCE (500-2400)	F F
54.	Liliaceae	4	Lilium nepalense	WCE (1430-4200)	F
55.	Verbenaceae	1	Lippia nodiflora	WCE (180-1630)	A
56.	Lauraceae	1			
57.	Lauracae	7	i. Machilus gamblei (Kathe kaulo) ii. M. odoratissima (Kaulo)	C (610-2130) WC (1520-2400)	F F
58.	Anacardiaceae	1	Mangifera indica	Cultivated in tropical and subtropicel region	A
59.	Labiatae	2	i. Mentha arvensis ii. M. sylvestris	Cultivated -	F F
60.	Magnoliaceae	4	i. Michelia champaca ii. M. kisopa	C (300-1500) C (1280-2130)	F F
61.	Labiatae	2	Micromeria biflora	WCE (500-3660)	F
62.	Dipsaceae	3	Morina longifolia	WCE (2290-3800)	F
63.	Myricaceae	1	Myrica esculenta (Kaphal)	WCE (1370-2650)	A

S.No.	Family	Total No. of species	Species reported to contain essential oil (Common/Vernacular name)	Distribution (Altitudes in Meters)	Availability
64.	Valerianaceae	1	Nardostachys grandiflora (N. jatamansi)	WC (3650-5300)	F
65.	Labiatae	5	Nepeta leucophylla	WC (2100-3500)	A
66.	Solanceae	2	Nicotiana tabacum	Cultivated in tropical and sub-tropical regions	A
67.	Labiatae	4	i. Ocimum basilicum	Cultivated in subtropical region	
			ii. O. sanctum	Cultivated in Tropical and subtropical areas.	
68.	Umbelliferae	3	-	-	-
69.	Oliaceae	2	Olea cuspidata	W (1100-2130)	F
70.	Labiatea	2	Origanum vulgare	WC (1800-3500)	F
71.	Oleacea	2	Osmanthus fragrans	C (1300-2300)	F
72.	Santalaceae	2	Osyris wightiana (Noon Dhiki)	WCE (700-2200)	A
73.	Pandanaceae	2	-	-	-
74.	Labiatae	1	Perilla frutescens (Seelam)	WCE (200-2140)	F
		1	-	-	-
75.	Pinaceae	2	i. Pinus roxburghii (chirpine Rani salla)	WCE (100-2200)	A
			ii. P. walliciana (Blue pine, salla)	WCE (1400-3900)	A
76.	Apocyanaceae	1	i. Plumeria acutifolia	Cultivated in subtropical region	
			ii. P. plebeium	WCE (300-2100)	F
77.	Rosaceae	1	i. Pyrus pashia (Mayal)	WCE (900-2400)	F
			ii. R. scleratus	WCE (280-2400)	F
78.	Polygonaceae	6	Rheum emodii (Padam Chall)	CE (2300-3600)	A
			-	-	-
79.	Rosaceae	4	i. Rosa macrophylla	WCE (1400-4700)	F
			ii. R. sericea	WCE (1700-4900)	A
			iii. R. webbiana	W (2300)	R
			Rubus. paniculatus	WCE (900-3000)	F
80.	Polygonaceae	4	Rumex nepalensis	WCE (900-3000)	F
81.	Buxaceae	3	Sarcococca hookeriana	WCE (900-3300)	A
82.	Saurauriaceae	3	Sauraulia napaulensis (Gogan)	WCE (700-2100)	A
83.	Umbelliferae	3	Selinum tenuifolium (Bhoot Kesh)	CE(2100-3900)	A

S.No.	Family	Total No. of species	Species reported to contain essential oil/ (Common/Vernacular Name)	Distribution (Altitudes in Meters)	Availability
84.	Pedaliaceae	1	Sesamum indicum	Cultivated in tropical and subtropical regions	A
85.	Dipterocarpaceae	1	Shorea robusta (Sal)	WCE (up to 1200)	A
86.	Rutaceae	3	Skimmia laureola	WE (2400-3000)	F
87.	Compositae	1	Sphaeranthus indicus	WCE (200-1100)	F
88.	Oleaceae				
89.	Myrtaceae	4	Syzygium cumini	WCE (180-1980)	F
90.	Compositae	3	Tenacetum vulgare	WC (2600-4700)	F
91.	Labiatae	1	Thymus serpyllum	C (2440-4100)	F
92.	Apocyanaceae	2	Trachelospermum T. repens	WC (300-2700) C (1300-2700)	F F
93.	Papilionaceae	4	-	-	-
94.	Pinaceae	1	Tsuga dumosa (Hemlock)	WCE (2100-3900)	A
95.	Malvaceae	1	Urena lobata	WCE (300-1980)	F
96.	Valerianaceae	2	i. Valeriana hardwickii (Nakali Jatamansi) ii. V. jatamansi (V. wallichii)	WCE (1200-4300) WC (1500-2600)	F F
97.	Gramineae	1	Vetiveria zizanioides	W	F
98.	Violaceae	8	i. Viola biflora ii. V. pilosa	WCE (1800-4000) WCE (800-2900)	F F
99.	Loranthaceae	3	Viscum album	W (600-2400)	F
100.	Verbenaceae	1	Vitex negundo	WCE (120-2100)	A
101.	Compositae	1	Xanthium strumarium	WCE (150-2400)	F
102.	Rutaceae	5	i. Zanthoxylum acanthopodium ii. Z. armatum (Z. alatum) (Timur) iii. Z. oxyphylum	WCE (1680-2600) WCE (900-2900) CE (2000-2770)	F A A
103.	Zingiberaceae	1	Zingiber officinale (Zinger, Aduwa)	Cultivated in tropical and sub-tropical regions.	A