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HIGH LEVEL ADVISORY ASSISTANCE TO STREAMLINE THE PROCESSING TECHNOLOGY OF "LITYAZOL CEMAL", A HERBAL PHARMACEUTICAL PRODUCED IN TURKEY

SI/TUR/93/801/11-01

TURKEY

Technical report: Findings, work performed and recommendations*

(First Mission)

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

Based on the work of M. B. Nareshima,
chemical technologist (plant engineer)

Backstopping Officer: T. De Silva
Chemical Industries Branch

* This document has not been edited.

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CONTENTS

ABSTRACT

The UNIDO expert on mission to Project DP/TUR/88/001/, along with NPD, Scientist and Engineer site visited Dr. Cemil Sener Laboratory, Manisa, near Izmir, during October 1991, at the personal request of the Managing Director of the Laboratory. The factory set up in a residential type of building in the year 1934, is reported to be the only company producing "Lityazol Cemil" an alcoholic extract of the root bark of the wild herb "Scolymus hispanicus". The liquid extract is effectively used against the "stone disorders" in the urinary track, and is being produced uninterruptedly since its inception in 1934, when it was registered as an ethical pharmaceutical by the Ministry of Health. Since then the process technology and the equipment remained unchanged and have become obsolete and uneconomical.

The management of the company requested the Director of MPRC, who through the Govetnment sought UNIDO assistance to update the process technology and modernise the process equipment. The MPRC had conducted laboratory experiments for the extraction of "Lityazol Cemil" from the root bark of Scolymus hispanicus using various concentrations of ethanol as well as distilled water. A process was developed for the extraction and identification of total triterpenic compounds and *[alpha]*-amyrin acetate.

A pilot plant based on the design drawings provided by the UNIDO expert [please refer his report, DP/ID/SER. A/1588, 22 July 1992 (DP/TUR/88/001)], was fabricated in the workshop of the university and installed at MPRC by the Counterpart engineer.

The expert on mission SI/TR/93/801/11-01 reviewed the laboratory results with the NPD, and counterpart Scientists and Engineer associated with the project, drew up a detailed programme to scale up operations and succesfully developed production technology, uptimising process conditions.

The following optimum conditions have been established:

- coarsely powdered, dried root bark could be used
- one is to four ratio of solid to solvent
- rectified spirit (96% ethanol) be used as solvent
- three percolators for3.5 hours each on counter-current principle on battery system
- that solvent losses are about 15%
- that the time to complete one batch is about 9-10 hrs.
- that the efficiency of the extraction is about 85-90%
- that the technology package developed is complete with design drawings of plant and equipment, analytical correlations and pharmacological studies for the activity of the ethanol extract.

INTRODUCTION

Dr. Cemil Sener Laboratory at Manisa, near Izmir, Turkey is reported to be the only company, producing "Lityazol Cemil" without proper quality controls, an alcoholic extract of the root bark of the wild herb "Scolymus hispanicus". The liquid extract is effectively used against the "stone disorders" in the urinary track, and is being produced uninterruptedly since 1934, the year when it was registered as an ethical pharmaceutical by the Ministry of Health Government of Turkey. Since then the process technology and the equipment remained unchanged, have become obsolete and uneconomical.

The production of "Lityazol Cemil" is a batch operation which takes 42 days and results in about 83% solvent losses (96% ethyl alcohol).

The company is currently using about 2 tonnes of dried material and about 2630 litres of 96% ethanol a year to produce annually about 2000 litres of product to no known quality, since no quality control facilities and trained personnel are available, except pH, viscosity and specific gravity measuring instruments.

The company approached MPRC and through the government UNIDO with a request to update process technology, modernize its production facilities and improve quality and quantity of the product to meet the local demand and for export to Europe.

Laboratory experimentation has been carried out by MPRC on a 10 litre pilot plant fabricated by it in the University's workshop to the designs provided by the UNIDO expert on mission to the project DP/TUR/88/001/ during 1991. Ref.Page-14

The UNIDO expert on mission to the current project No. SI/TUR/93/801/11-01 titled "High level advisory assistance to streamline the processing technology of "Lityazol Cemil", a herbal pharmaceutical produced in Turkey, is mandated to generate the following outputs:

A detailed report outlining the improved technological process and quality specifications of "Lityazol" and recommendations on increasing production through:

- reduced batch operation period from 42 days to 1/4 of the present duration,
- reduced solvent losses from the current level of 83%,
- improve the economy of production and marketing for local and eventual foreign market.

The expert will be specifically required to work in collaboration with counterpart staff at Sener Factory and Anadolu University in carrying out the following duties:

1. Survey the site for Lityazol production and work out layout.
2. Survey sources for equipment production.

3. Prepare a list of required equipment after developing the process.

4. Conduct pilot runs and testing on Lityazol products at Anadolu University.

5. Develop process technology and process control parameters for improvement of production of "Lityazol" at MPRC.

Job description attached (Annex I).

Present Set-up

The Laboratory set-up in a residential type of building in the year 1934 has percolation, concentration and bottling, labelling, packing sections as well as a small quality control unit.

The percolation section has 15 tin coated copper percolators of about 20 kg capacity each, an equal number of enamelled cans, a PVC plate and frame filter press and a 2m3 miscella storage tank.

An electrically heated 100 litre capacity distillation still with goose neck and a coiled condenser. A small stainless steel electrically heated, open top, concentrator completes the concentration section.

Additionally, the laboratory has an electrically driven hammer mill for size reducing, the sun dried root bark.

The extraction operation that is being followed in the laboratory is, that 20 kg of coarsely crushed root bark is mascerated with 20 litres of ethanol(70%) for 24 hrs and then the percolate collected at the rate of 70 drops per minute. Percolation continued with additional five lots of 20 litres each of ethanol (70%).

The first 20 litres of percolate is kept aside and the subsquent percolates mixed together and distilled in electrically heated distillation still.

Roughly about 450-500 litres of ethanol (40%) is obtained when 1500 litres of miscella is distilled. The concentrate thus obtained is boiled in the electrically heated stainless steel vessel of about 15 litre capacity, till the mass becomes viscous. This concentrate is then mixed with 20 litre first percolate, set aside earlier, filtered in the filter press.

Only physical characteristics Viz., pH, viscosity and density of the product is measured before bottling.

The following physical characterization is generally observed:

	Min	Max
pH	6.0	4.5
Viscosity	2.0 cp	4.5 cp
Density	0.93 gr/ml	0.99 gr/ml

ACTIVITIES

The expert on mission visited the laboratory at Manisa along with the counterpart engineer, held discussions with the Managing Director, Commercial Manager, and Chemical Engineer of the Laboratory.

The Managing Director, informed that the laboratory is currently processing an average of about 2 tonnes of sun dried Scolymus hispanicus root bark collected from the wild to produce "Lityazol Cemil", and is planning to plant on 200 acres of about 750 acres of his private farm, while continuing the exploitation from naturally grown sources. At later stage, he may consider extending cultivation to about 400 acres.

It appears that the plant takes about 2 years to mature and ready for harvest.

He was quite optimistic and sure of collecting 10-15 tonnes per annum of the sun dried root bark by 1995 and expressed a desire that the new plant when installed should be able to process all of it in a year.

UNIDO expert accompanied by the M.D. and counterpart engineer visited the nearby port city of Izmir, to survey, the facilities and expertise available in the fabrication of chemical plant and equipment in stainless steel. The machine shops visited have no previous experience to undertake such jobs, but have experience in the manufacture of mechanical washing, filling and sealing of bottles of various sizes and shapes.

This expertise available in Izmir could be suitably used in building new mechanised filling section.

The opportunity was also utilised in discussing the location of the new plant. The following alternatives were considered:

- the Laboratory building after suitable modification to the existing building. M.D. agreed to consult local civil engineers in this regard.

- a new construction with in the Laboratory compound

- to procure a suitable but readily available building in the industrial estate

- a new construction on an all together new site.

- approximate space requirements to house the new plant has been provided to M.D.

The Expert on mission fielded from 24 Nov 93 to 6 Jan 94, has carried out the following functions in collaboration with counterpart scientists and engineer of MPRC Eskisehir:

1. Reviewed laboratory level experimental work conducted at MPRC.

2. Planned and systematically conducted scale-up operations on 9 litre 3-stage counter-current percolator system (Battery).

3. Succesfully optimised process conditions and developed process technology.

4. Drastically cut down batch time from 42 days to about 9-10 hours.

5. Low solvent losses. About 15% compared to 83% losses at the factory. This can be reduced further to about 5% at the factory in the new set-up.

6. Higher yields of product; 3 litres per kg of root bark as against 1 litre/1 kg currently produced at the company.

7. Very high concentration of triterpenic compounds particularly *[alpha]* -amyrin acetate in the extract. Nearly 20 times higher concentration of the later in the extract, compared to the company produced "Lityazol Cemil".

8. Higher efficiency of extraction Viz., about 85-90%.

9. Less impurities like tannins, gums, sugars etc., in the ethanol.

10. Pharmacological studies showed that *[alpha]*-amyrin acetate is the most active component in the extract.

11. Worked out design parameters and design drawings for a commercial plant based on pilot plant experimentation.

12. The new production plant, when implemented will have the capacity to process about 2.5-3.0 tonnes of root bark per annum, when operated one shift a day, however, the same plant would be able to process about 12-15 tonnes per annum when operated on 24 hrs. a day and 300 days per annum.

13. Pharmacological test results. Please refer Annex IIIa, IIIb

14. Two pharmacology teams, one from TBAM and another from Medical Faculty of Osmangazi University, who independently investigated the drug's activity have reported that two times dilution of the pilot plant extract has the same activity as that of the "Lityazol Cemil", this in other words means that the produce from the pilot plant is three times and double potency to that produced in the factory per kg of root bark.

15. These reports clearly indicate that the ethanolic extract obtained from the percolators after removal of suspended solid particles in the filter press and diluting twice with distilled water is ready for use in the filling section.

16. The operations of distillation of miscella and boiling the residue, that is currently practiced in the factory automatically become redundant.

17. Surveyed the availability of expertise to undertake fabrication of chemical plant and equipment to the designs provided by the Expert on mission.

18. Development of analytical techniques and correlations, which would act as ready reckoner to read out dry matter, TTC and *[alpha]*-amyrin acetate based on UV Spectrometry, Refractive Indices etc. Please refer Annex IV.

19. General plant layout. Annex V

20. Flow diagram-production of "Lityazol Cemil" from Scolymus hispanicus based on new technology. Annex VI

21. Design drawings of all the equipment have been drawn and appended, Annex VII

CONCLUSIONS

It is concluded that:

1. As a result of the current studies conducted at pilot plant level, it is echnically feasible and economically viable to produce modified Lityazol from the root bark of Scolymus hispanicus at the industrial level based on technology package developed during the mission.

2. It is possible to produce an alcoholic extract from the root bark Scolymus hispanicus to reproducible quality.

3. It is technically feasible to increase the capacity of the suggested plant from 2.5-3.0 tonnes of root bark per annum to 12-15 tonnes per annum, by increasing the hours of operation from 8 hrs a day to 24 hrs/day and 300 days per annum.

4. It is possible to produce an alcoholic extract containing higher concentration of triterpenic compounds and *[alpha]*-amyrin acetate.

5. The extract after diluting with an equal amount of distilled water has the same activity as that of factory produced "Lityazol Cemil". Ref.Annex IIIa IIIb.

6. The yield of pharmacologically active extract comparable with that of Lityazol will be three times per kg of root bark and double potency, to that produced in the factory.

7. The distillation and boiling operations currently being followed by the factory at Manisa, will become superfluous.

8. The capital investment and running cost of the new plant when implemented will be correspondingly reduced. Thus increasing profitability.

9. It is feasible to produce the extract to national and international standards.

RECOMMENDATIONS

1. Collaboration with MPRC on a long-range basis for "trouble shooting" in the production line, quality control, pharmacological tests for proper dosage and product diversification.

2. Every batch of product produced at the factory should be analysed completely and the components shown clearly on the bottles and packings.

3. The company and MPRC may consider, to diversify the production, to utilise the spare capacity, during the lean period.

4. It is qenerally considered a sound economical policy to base a factory on multiple products rather than on a single product.

5. The management of the company may consider recruitment of production engineers and analytical chemists at least one each before project implementation stage and have then trained at MPRC and associate them during erection and commissioning of the plant and equipment. This when done would prepare then to undertake production operations with confidence and competence.

6. The company should have minimum of quality testing instruments Viz., UV, Refractometer, TLC-Densitometer.

7. Messers Seker Makina Eski/ehir possesses extensive infrastructure and expertise to undertake fabrication of the plant and equipment in stainless steel, provided suitable design grawings are supplied. In the past this company as done a good job in the fabrication of some pilot plants Viz., essential oils fractionation column, distillation stills, condensers, 2m3 steam distillation plant. This party may be considered for getting the proposed new set-up fabricated for the production of "Lityazol Cemil".

8. Follow-up action required on first mission in preparation for the second mission which is due to take place in the second quarter of 1994. Some experiments and trials should be done by MPRC counterparts with a view to ensure quality of the product and check the effect of moisture content in the root bark on the quality of the product.

9. Follow-up on the local fabrication of the proposed plant, for which necessary designs have been provided.

10. To make arrangements to set-up a quality control laboratory.

11. The product obtained at MPRC may be handed over to the company for clinical tests to be conducted through their normal channels.

ACKNOWLEDGEMENTS

The author acknowledges with thanks Dr.Tuley De Silva, Special Technical Adviser Chemical Industries Branch, UNIDO Vienna, Dr. K.Hussein. UCD,UNDP, Ankara, Mr. Rasit Karaosmanoglu, Managing Director of Dr. Cemil Sener Laboratory, Manisa for their generous help and advise.

Prof.Dr.K.H.C.Baser, Director MPRC, who placed whole heartedly all the facilities of MPRC, as well as the needed scientific and technical members of MPRC at the disposal of the UNIDO Expert, with his whole hearted support it has been possible to develop technology package. I acknowledge his help and advise with thanks.

I also acknowledge with thanks Doç.Dr.Nese Kirìmer. Ms. Zeynep Toprakli for quality analysis and for making laboratory data available, Mr. Sedat Hakkì Beis, the young Chemical Engineer, for assistance in pilot plant experimentation and plant drawings. Prof.Dr.Yusuf Öztürk, Mr. Süleyman Aydìn, Ms. Rana Beis, and Doç.Dr. M. ipek Cingi for pharmacological tests, Ms. Berrin Bozan for the assistance in the development of quality correlations and Ms. Berna Bozan for typing the report.

Annex 1

UNIDO

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

JOB DESCRIPTION

SI/TUR/93/801/11-01

Post Title: Chemical Technologist (Plant Engineer)

Duration: 3.0 m/m (with split mission to assure follow-up to recommendations perhaps 6 months after initial mission) 1.0 m/m First mission

Date Required: ASAP

Duty Station: Eskisehir and Manisa in Turkey, and travel in country as appropriate

Purpose of Project: High level advisory assistance to streamline the processing technology of "Lityazol Cemil", a herbal pharmaceutical produced in Turkey.

Duties: The expert will be specifically required to work in collaboration with counterpart staff at Senel Factory and Anadolu University in carrying out the following duties:

1. Survey the site for Lityazol production and work out Layout.
2. Survey sources for equipment production.
3. Prepare a list of required equipment after preliminary process.
4. Conduct pilot runs and testing on Lityazol products at Anadolu University.
5. Develop process technology and process control parameters for improvement of production of "Lityazol" at MPRC.
6. Introduce good manufacturing practices safety requirements, and marketing techniques

Finally, the expert will furnish a report embodying the progress made and outlining his recommendations to both UNIDO and the Government.

Qualifications: Chemist/Pharmacist or chemical engineer with experience in the processing of essential and fixed oils from plant materials at pilot and industrial levels, and marketing experience is desirable

Language: English

Applications and communications regarding this Job Description should be sent to:

Project Personnel Recruitment Section, Industrial Operations Division

UNIDO, VIENNA INTERNATIONAL CENTRE, P.O. Box 300, Vienna, Austria

Annex 2

Report

Laboratory Experiment

MPRC has developed a laboratory scale process for the extraction of Scolymus hispanicus after detailed experimentation in the laboratory.

It is as reported elsewhere is that, finely powdered root bark is boiled in ethyl alcohol of various concentrations as well as in distilled water for periods of 2,4,6 and 8 hours and found that boiling in 70% ethanol for 4 hours gave best results. The extract that obtained is filtered and analysed for dry matter, triterpenic compounds, expressed as butanol extractables and α-amyrin acetate and compared with that of factory produced "Lityazol Cemil".

These samples as well as Lityazol Cemil were tested on mice and rats for pharmacological activity, and found that α-amyrin acetate is the most active compound of triterpenic compounds pharmacologically.

The results:

	Solvent Ethanol %	Dry matter %	Triterpenic compounds (BuOH extn) %	α-amyrin acetate %
Powdered root bark	96	10	4.5	0.3
	70	29	4.0	0.15
	50	39	2.8	Traces
	water	53	2.5	Traces
Lityazol Cemil	-	10	2.04	0.01

Finely ground root bark boiled in different concentrations of ethanol for periods, indicated below:

Extraction time Hrs	96%	70%	50%	Distilled water
2	9.0	27.4	34.7	47.3
4	9.9	28.8	38.8	53.4
6	9.6	27.8	38.2	52.8
8	9.9	28.6	37.2	53.4

% Triterpenic compounds, % α-amyrin acetate in the ethanol extract.

Ethanol	Dry matter	Butanol extract %	α-amyrin acetate
96%	10	4.5	0.3
70%	29	4.5	0.15
50%	39	2.8	Very little
Distilled water	53	2.5	Very little
Lityazol	10	2.04	0.01

Pharmacological Studies

Sample	Conc. in bath of dry matter	Effect	
EtOH	1.2 mg/ml	↓ 8.7 ± 4.8 ↑ 17.2 ± 8.6	
BuOH	1.0 mg/ml	↑ 15.2 ± 1.8 ↓ 10.1 ± 3.7	
Lyophilised Lityazol	1.2 mg/ml	↓ 9.4 ± 1.9	
α.a.a.	0.1 mg/ml	↑ 5.2 ± 1.5 ↓ 45.8 ± 4.2	after acetylcholine

v relaxation activity
^ contraction activity

Pilot Plant Experiments

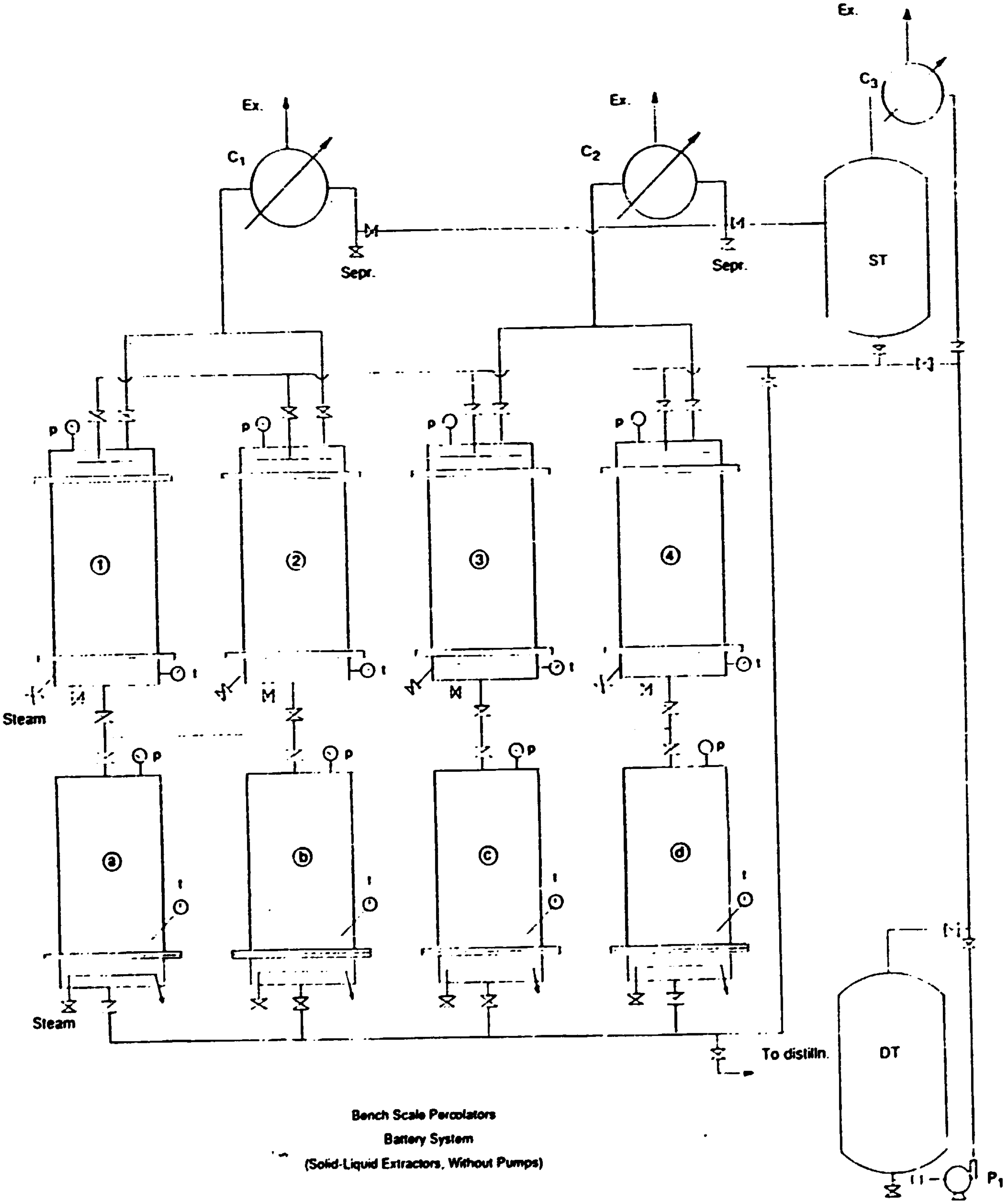
Keeping in view continuous production activities at the company and lack of trained analysts and analytical instruments, the programme was so drawn that simultaneously with optimisation studies at pilot plant level, to develop simple correlations to determine triterpenic compound and α-amyrin acetate, based on UV and Ref. indices of the corresponding extracts of the root bark.

The general sketch of pilot plant set-up of MPRC as shown in the enclosed drawing consists of four percolators of 10 litre each (1.5 kg of crushed root bark per batch) filted with internal steam coil for preheating and live steam point for stripping of marc. Percolators are connected to each other in such a way that miscella can be circulated from any one percolator and fed into any one of the remaining three percolators to increase the filexibility of operations, with a view to optimum utilisation of the pilot plant as a whole. This is the reason as reported elsewhere that if the commercial plant when operated for 24 hrs a day and 300 days a year, the production capacity could be increased to 12-15 tonnes a year compared to 2-3 tonne a year, if the plant is operated for 8 hrs a day.

Actually three percolators are considered sufficient for the optimum extraction values, fourth one is provided to continue the extraction without break, when one of the three percolators are under stripping and evacuation.

The following variables have been studied at the pilot plant level (1.5 kg/batch) worked on counter current (battery system) to arrive at optimum conditions are given below:

- Particle Size : Mixed
- Solvent : 96% ethyl alcohol
- Solid to Solvent Ratio : 1:6.5 ; 1:5 ; 1:4
- Time : 1,2,3,3.5,4 hrs
- Temperature : Ambient temp 17°C
  45°C-50°C



Bench Scale Percolators
Battery System
(Solid-Liquid Extractors, Without Pumps)

1,2,3,4	.. Percolators	t	.. Thermometer
a,b,c,d	.. Miscella tanks	p	.. Pressure gauge
C_1, C_2, C_3	.. Condensers	DT	.. Distillation Tank
P_1	.. Solvent pump	ST	.. Solvent tank

Designed by: M. B. NARASIMHA, S. H. BEIS
Anadolu University Medicinal Plants Research Centre, Eskişehir, October 1991

1. Solid to Liquid Ratio 1:6.5

Charge : 1 kg
(F2) Solvent : 6.7 L

Min	°C	gr/100 ml	Flow Rate
60	50	1.50	5.4
120	50	1.60	5.4
150	48	1.62	5.1
180	55	1.70	5.3
210	50	1.69	5.2

Product: 4.7 L

(F3) Solvent: 4 L (0.36%)

Min	°C	gr/100 ml	Flow Rate
0	40	0.36	5.4
60	50	0.92	5.1
120	52	0.94	5.2
150	48	1.02	
180	55	1.06	4.8

(F2) Miscella : 3.0 L

(F4) Solvent : 5 L (ethanol 96%)

Min	°C	gr/100 ml	Flow Rate
0	45	0	
60	52	0.38	5.4
120	48	0.40	
150	50	0.44	
180	53	0.50	5.4

(F3) Miscella : 4.3 L

2. Solid to Liquid Ratio 1:5

Charge: 1.5 kg (0.25 fine, H_2O 9%)
Solvent: 7.5 L (%1, 242 F2)

Min	°C	gr/100 ml	Flow Rate
0	45	1.0	4.9
60	50	1.50	5.1
120	50	1.72	5.2
150	45	1.76	5.0
180	50	1.82	5.1
210	50	1.88	5.1

F1 Product: 5.8 L

F3 Solvent: 6 L (0.45%)

Min	°C	gr/100 ml	Flow Rate
0	50	0.45	5.4
60	50	0.86	
120	45	1.04	
150	50	1.04	
180	50	1.10	5.2

F2 Miscella: 5.7 L

F4 Solvent : 6 L (ethanol 96%)

Min	°C	gr, 100 ml	Flow Rate
0	48	0	5.0
60	50	0.36	
120	50	0.42	5.1

F3 Miscella: 5.9 L

3. Solid to Liquid Ratio 1:4

Charge: 1.5 kg

Solvent: 5.8 L (1.10%)

Min	ºC	gr/100 ml	Flow Rate
0	40	1.10	1.2
60	50	1.84	1.4
120	48	2.24	
150	52	2.36	1.3
180	50	2.38	1.2

F1 Product : 3.85 L

F3 Solvent: 5.8 L (0.42%)

Min	ºC	gr/100 ml	Flow Rate
0	40	0.42	1.8
60	50	1.06	
120	50	1.14	1.6
180	52	1.26	1.8

F2 Miscella: 4.8 L

F4 Solvent: 6 L (ethanol 96%)

Min	ºC	gr/100 mi	Flow Rate
0	40	0	1.8
60	50	0.7	
120	48	0.8	1.8

F3 Miscella: 5.4 L

4. Solid to Solvent Ratio 1:4

Charge: 1.5 kg

F2 Solvent: 6 L (1.2%)

Min	°C	gr/100 ml	Flow Rate
0	40	1.1	2.8
60	48	-	
120	49	-	3.1
180	49	2.52	3.0

F1 Product: 3.4 L

F3 Solvent: 6 L (0.96%)

Min	°C	gr/100 ml	Flow Rate
0	40	0.96	3
60	51	-	
120	52	-	
180	49	1.68	3

F2 Miscella: 3.7 L

F4 Solvent: 6 L (ethanol 96%)

Min	°C	gr/100 ml	Flow Rate
0	45	0	3.6
60	50	-	
120	48	0.84	3.6

F3 Miscella: 6 L

5. Solid to Solvent Ratio 1:4

Charge: 1.5 kg

Solvent: 6 L (0.84%)

Min	°C	gr/100 ml	Flow Rate
0	40	-	4.8
60	48	-	
120	50	-	5.0
130	52	2.14	
210	49	2.26	4.9

F1 Product: 4.7 L

F3 Solvent: 6L (0.87%)

Min	°C	gr/100 ml	Flow Rate
0	45		4.8
60	50		
120	52		
180	50	1.51	5.1
210	48	1.52	5.0

F2 Miscella: 5.4 L

F4 6 L (%96)

Min	°C	gr/100 ml	Flow Rate
0	45		5.1
60	50		
120	50		
180	53	0.60	5.2
210	50	0.60	

F3 Miscella: 5.75 L

6. Solid to Solvent Ratio 1:4 Cold Percolations

Charge: 1.5 kg

Solvent: 5.8 L (1.42%)

Time	°C	gr/100 ml	Flow Rate
9^{10}	18	1.42	interrupted
12^{10}	18	1.84	

Miscella: 4.15 L (1.84%)

Solvent: 5.8 L (0.6%)

Time	°C	gr/100 ml
13^{25}	18	0.6
16^{25}	18	1.00

Miscella: 5.0 L (1%)

Solvent: 6 L (ethanol 96%)

Time	°C	gr/100 ml
16^{50}	18	0
18^{50}	18	0.8

Miscella: 5.4 L (0.8%)

7.

Charge: 1.25 kg

Solvent: 5 L (1.0%)

Time	^oC	gr/100 ml	Flow Rate
$8.^{5}$	18	1.0	4
$11.^{45}$	18	1.32	

Miscella: 4.5 L (1.32%)

Solvent: 5.4 L (0.32%)

Time	^oC	gr/100 ml	Flow Rate
$12.^{00}$	18	0.32	4
$15.^{00}$	18	0.66	4

Miscella: 5.2 (0.66%)

Solvent: 5 L (90%)

Time	^oC	gr/100 ml	Flow Rate
$16.^{00}$	18	0	4
$18.^{00}$	18	0.17	4

Miscella: 4.9 (0.17%)

Pilot Plant Results

	Dry Matter	TTC	α.a.a. %
P.P. Expt.	%	from dry matter %	
1	1.88	44.7	0.186
2	2.38	50	0.19
3	2.48	37.9	0.193
4	2.46	47.97	0.23
5	1.38	44.93	-
Lityazol	10.5	38.6	0.01

1,2,3,4: Solid:Liquid 1:4

Temp: 45°C

Time: 3.5 hrs

Three stage battery type percolators system.

5: Same as above

Temp: Ambient temp - 17°C

The presence of the following compounds have been identified in the samples of ethanol extract from the root bark and the factory produced extracts:

	Root Bark extract Pilot Plant	Lityazol Cemil
Tannins	+	+
Sugars	+	+
Alkaloids	-	-
Anthracene glycosides	-	-
Triterpenic saponins	+	+
Flavone glycosides	-	-
Gum	+	+
Starch	+	-

Annex 3

Biphasic (Contractile & Relaxant) Effects of "Lityazol Cemil" on Rat Ileum in Vitro+

	Contraction (Mean)	Relaxation (Mean)
Acetylcholine	49.25 ± 4.94 (n=10)	0*
Lityazol Cem	11.75 ± 0.96 (n=8)	5.38 ± 0.24 (n=8)
Sample 1 (Pilot plant product)	11.63 ± 0.76** (n=8) F=1.61 P>0.10	5.38 ± 0.66*** (n=8) F=0.66 P>0.10
Sample 2 (Pilot plant product)	8.14 ± 0.64** (n=7) F=2.91 P>0.10	4.57 ± 0.65*** (n=7) F=2.19 P>0.10

*Rat ileum was standardized according to the contractile responses to acetycholine, as it has only contractile response on this tissue

Statistical differences between the responses to Lityazol Cemil and those to pilot plant products, which were diluted two-times with water, were evaluated by the Student's t-test (P values) and Analysis of variance (F values).

According to the results of the statistical comparison, there were no differences between Lityazol Cemil and diluted pilot plant products. This finding suggest that the samples tested are at least two-fold active than Lityazol Cemil.

+<u>Methodological reference:</u> Altan, V.M., Oztürk, Y., Yildizoğlu-Arı, N., Nebigil, C., Lafçı, D., Özçelikay, A.T.: Insulin action on different smooth muscle preparations., *Gen. Pharmacol.* 20(4): 529-535 (1989).

31.12.1993

Report

We studied on rat ileum. According to the results of the statistical comparison, there were no differences between Lityazol Cemil and 2,5 times diluted (with water) Pilot Plant Products.

Osmangazi University
Medical Faculty
Dept. of Pharmacology
Meşelik-Eskişehir-Türkiye

Annex 4

Correlation of TTC Content in the Ethanol Extract of *Scolymus Hispanicus*

Extensive search made in the library and computer data bases has indicated a total absence of methods of analysis and identification of active principles present in the drug, laboratory extracts and the factory produce. Hence it became a compulsive need to develop the appropriate techniques necessary for the identification of total triterpenic compounds and α-amyrin acetate, which the scientists MPRC have admirably achieved.

1. Total solid percentage in the ethanolic extracts by refractometer

1.1. Zero point calibration

Abbe Refractometer which works on the basis of water was recalibrated with ethanolic solutions.

The data obtained is as given in Table 1.

As shown in Figure 1, the zero point of 96% ethanol is similar to those values from 70 % to 96 %, and therefore they could be used for the estimation of the percentage of total solid of ethanolic extracts (EE).

1.2. The relationship between the percentage of dried matter and the percentage of total solid as determined gravimatically and by refractometer, respectivelly.

Eighteen samples of ethanol extracts obtined at pilot plant were prepared gravimetrically in the range of 0.13-5.2 as percentage dried matter. Each sample was measured by a refractometer as the percentage of total solid in the extracts (EE).

The percentage of total solid was calculated based on the zero point of 96% ethanol. The data obtained were as shown in Table 2. The relationship between the % of dried matter and the % of total solid (RI) was obtained graphically (Figure 2). The correlation coefficient r^2 is 0.997 and the equation is $y = 0.84167x + 0.10285$.

3. Determination of the amount of Triterpenic compounds (TTC) in the extracts obtained gravimetrically

Known amount of ethanolic extracts were evaporated by a rotary evaporator at 60 °C. The residue was dissolved in water : Butanol (50:50), and all the TTC were extracted by BuOH. The extraction time was 2 minutes and this procedure was repeated 4 more times. All the BuOH phases were collected in a flask and the BuOH was removed under vacuum at 60°C.

The residue was weighed, and the amount of TTC in the ethanolic extract was determined.

4. Determination of amount of TTC in the ethanolic extracts by Spectroscopic method (UV).

32.2 mg Butanolic extract which was used as a standard was dissolved in 25 ml of $CHCl_3$:MeOH (1:1) in a volumetric flask. This solution was used as a stock solution. Six dilutions in the range of 0.06 mg/ml to 0.4 mg/ml (0.5/10, 1/10, 1.5/10, 2/10, 2.5/10 and 3/10 ml, respectivelly) were prepared from the stock solution to obtain the calibration curve. Absorbance values were obtained by UV at 237 nm.

The data for calibration curve was given in Table 3.

The calibration was prepared by plotting the absorbance versus the concentration (Figure 3). The correlation coefficient, r^2, is 0.999. The calibration equation is $y= 2.1367x + 5.46 \cdot 10^{-3}$.

(y= absorbance at 237 nm, and x= concentration in mg/ml)

Using this calibration curve the percentage of TTC in the ethanolic extract with the % dried matter of 0.1 to 4 % were determined by measuring the absorbance (Table 4).

The calibration curve was drawn by plotting the % of dried matter versus the % TTC in the extract., The best correlation coefficient (r^2) obtained is 0.999. The equation of this curve is $y=0.76176x-4 \cdot 1769.10^{-2}$. (Figure 4).

The extracts were diluted to obtained the absorbance values between 0.2-0.8. The dilution factors were as shown in Table 5.

These results which were obtained by UV were compared with gravimetric method (Table 6). As shown in Tablo 6, the results obtained by UV and gravimetric methods were similar. Therefore this method is more suitable for the determination of % TTC than the gravimetric method because of its simplicity, rapidity and reproducibility.

Table 1.

% Ethanol	RI (Total solid %, zero point)
0 (water)	0
5	1.60
10	3.25
20	6.80
30	11.00
40	14.00
50	16.80
60	18.25
70	19.4
75	19.7
80	20.2
85	20.1
90	20.1
96	19.5

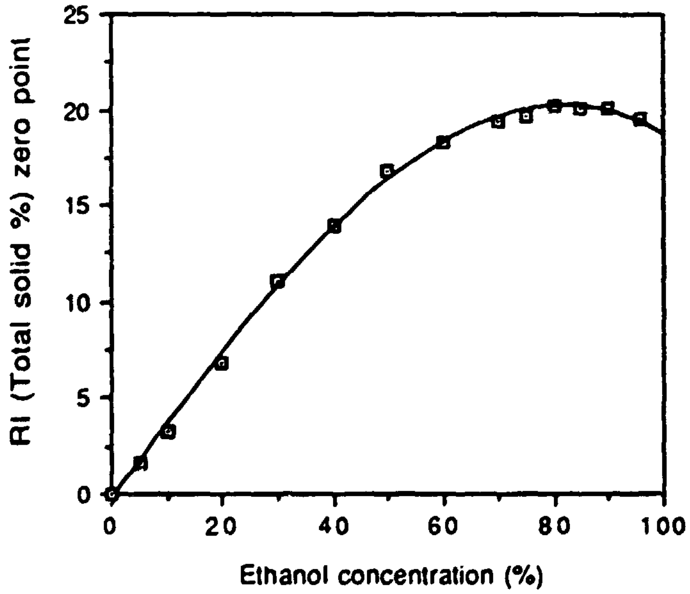


Figure 1.

Tablo 2.

Dried matted % (g/100 ml) by gravimetric method, S246	Dried matter % (g/100 ml) (RI, Total solid %) , S246
0.13	0.1
0.24	0.2
0.26	0.3
0.39	0.4
0.48	0.5
0.52	0.5
0.65	0.7
0.78	0.8
0.91	0.9
1.04	1.1
1.3	1.3
1.56	1.5
1.95	1.8
2.6	2.1
3.25	2.8
3.9	3.4
4.55	3.9
5.2	4.4

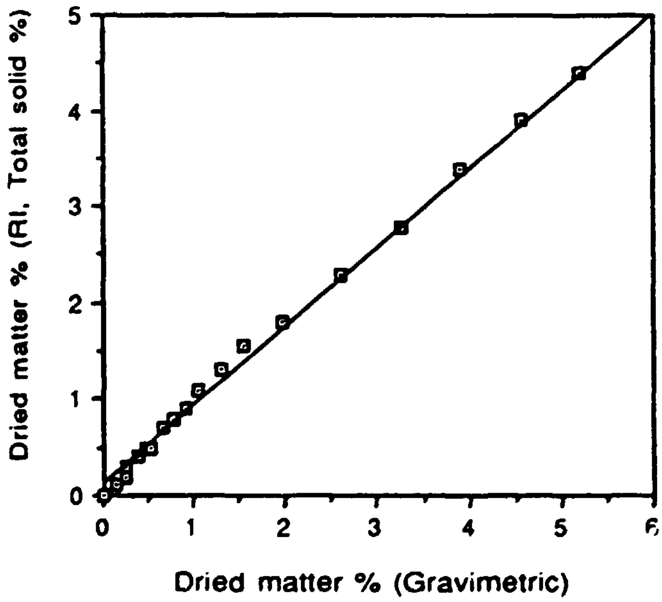


Figure 2.

Table 3

Dilution (ml/ml)	Concentration (mg/ml)	Absorption (237 nm)
0	0	0
0.5/10	0.0642	0.133
1/10	0.1284	0.285
1.5/10	0.1926	0.427
2/10	0.2568	0.566
2.5/10	0.3210	0.698
3/10	0.3852	0.810

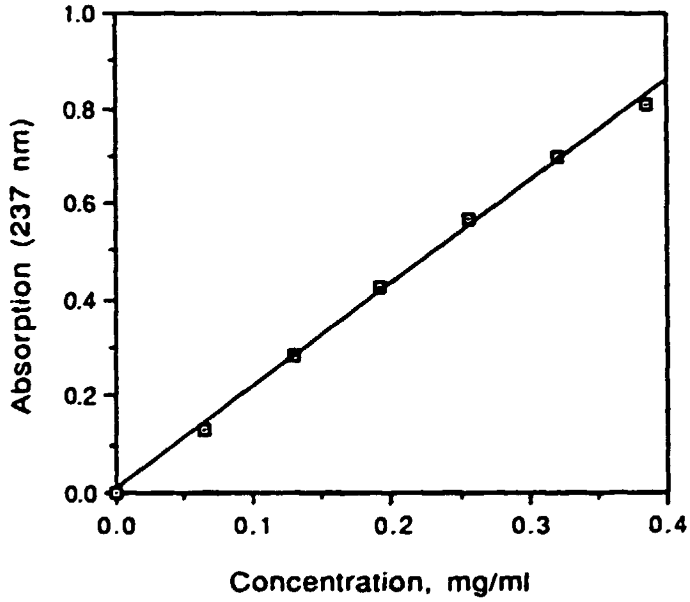


Figure 3.

Table 4.

% of Dried matter (g/ 100ml) (S246)	% of TTC (g /100 ml) (S246)
0.13	0.07571
0.26	0.1196
0.39	0.2226
0.52	0.3022
0.65	0.4698
0.78	0.5505
0.91	0.6569
1.04	0.7635
1.3	0.9817
1.56	0.1338
1.95	1.4192
2.6	2.009
3.25	2.4206
3.9	2.9047

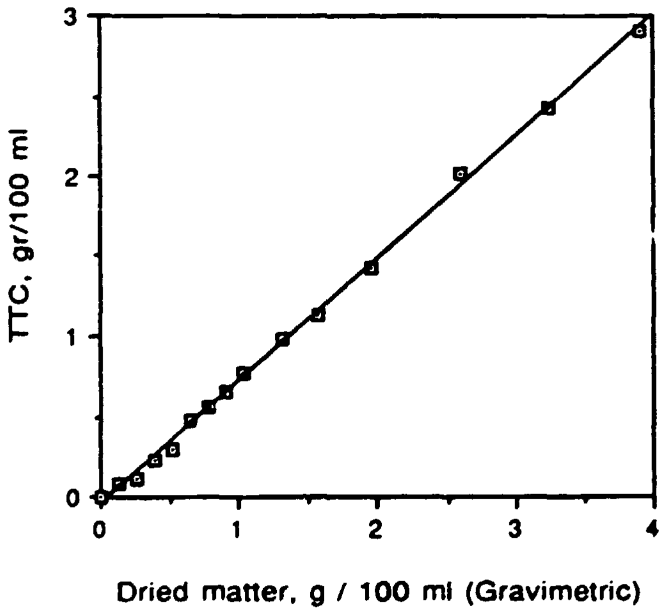


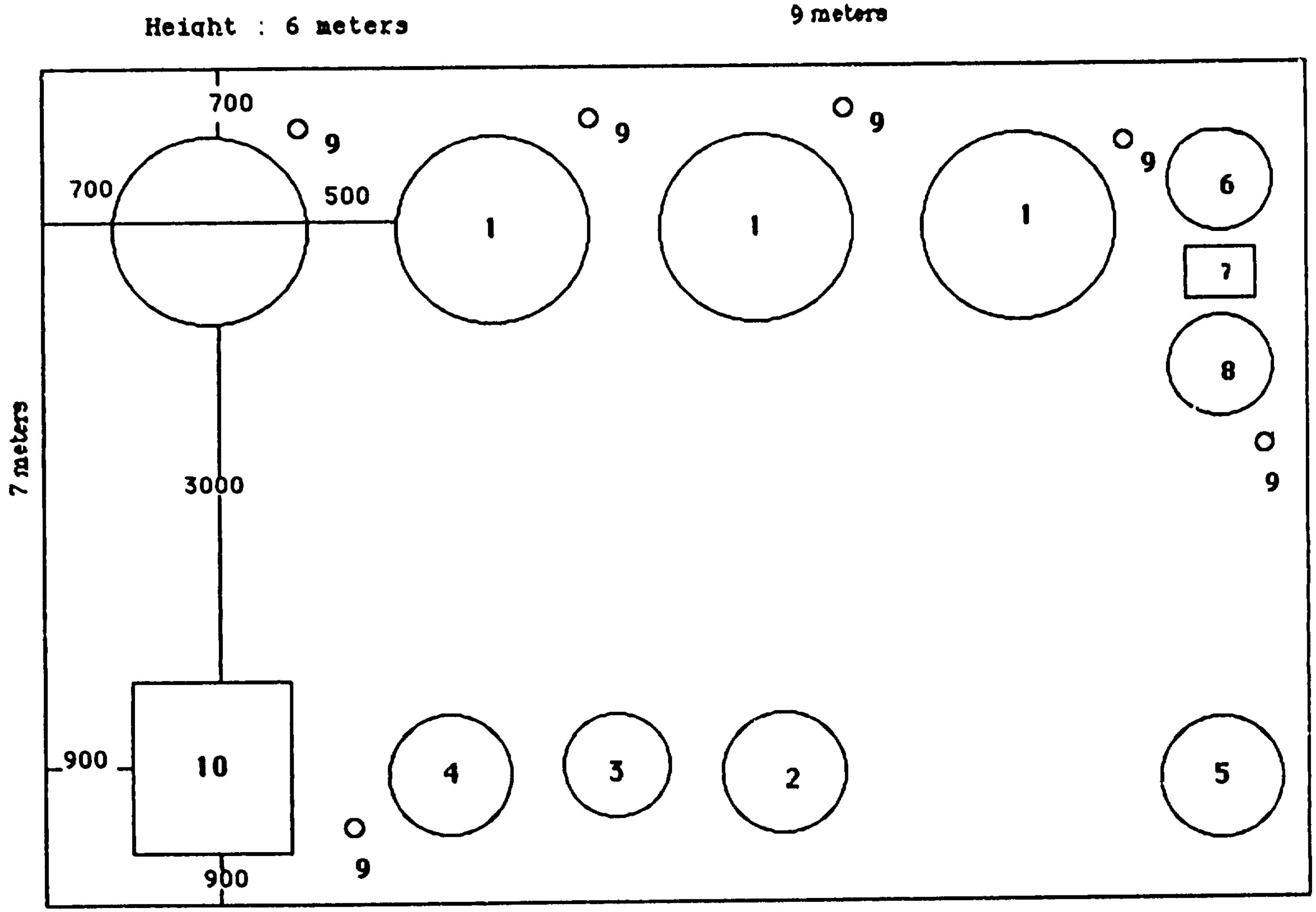
Figure 4

Table 5

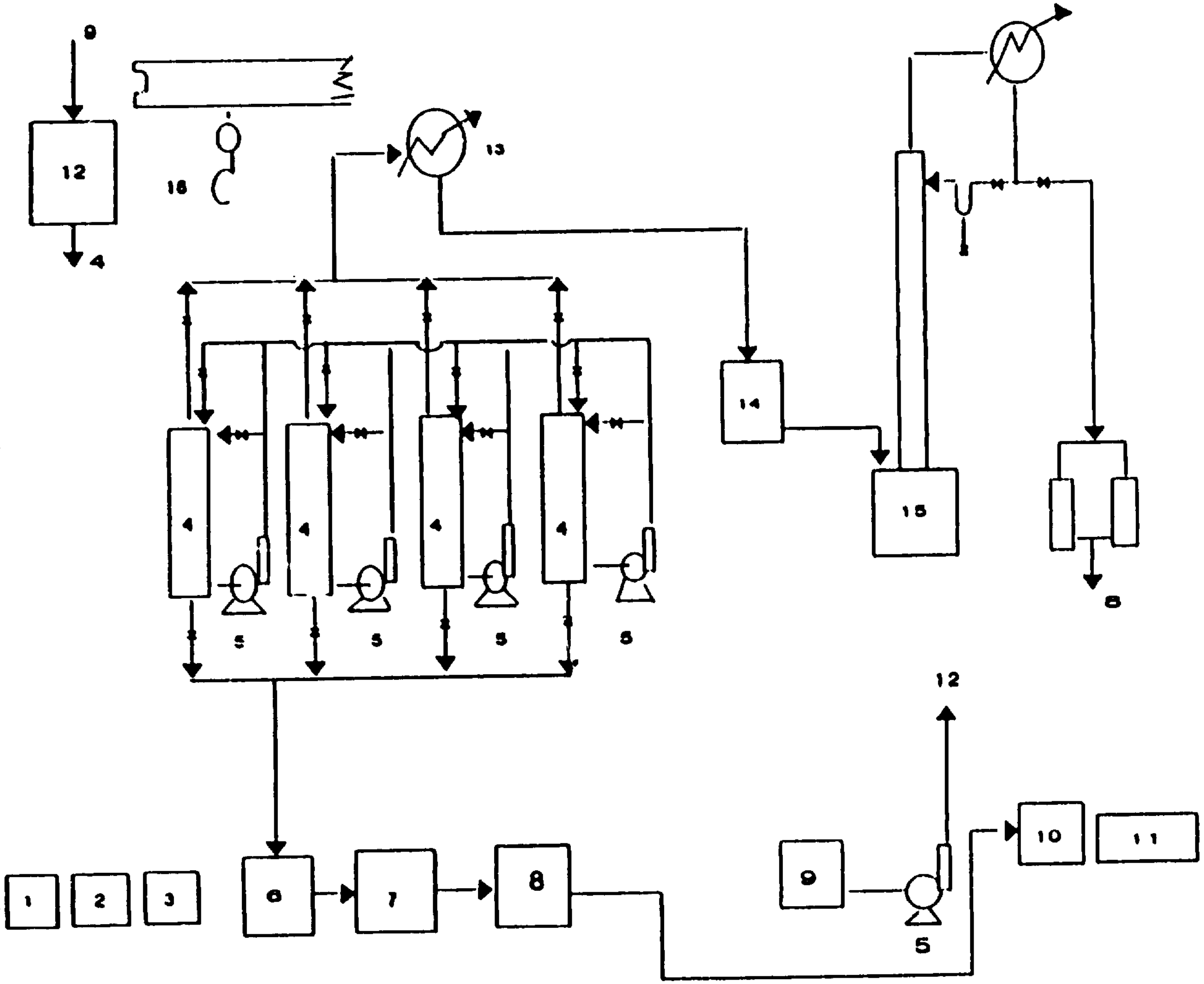
% of Dried matter (gr/100ml)	Dilutions (ml/ml)
0.1-0.2	10/25
0.2-0.6	5/25
0.6-1.0	1/25
1.0-2.0	0.5/25
2.0-4.0	0.2/25
4.0-5.0	0.1/25

Table 6.

% Dried matter (gr/100 ml)	% TTC (gr/100 ml) by UV	% TTC (gr/100ml) by gravimetric method
1.025	0.687	0.6839
1.219	0.820	0.789
1.516	0.984	0.9455
0.8052	0.604	0.5397



1. Percolator
2. Dilute alcohol tank
3. Rectification column
4. Rectified alcohol tank
5. Blender
6. Miscella tank
7. Filter press
8. Filtered miscella tank
9. Pump
10. Alcohol tank



16 Overhead Pully block	one
15 Rectification Column	one
14 Storage Tank (dil Ethanol)	one
13 Condenser	one
12 Ethanol (OH) Tank	one
11 Lityazol Bottling Packing	one
10 Lityazol Blender	one
9 Ethanol Storage	one
8 Filtered Miscella Tank	one
7 Sparkler Filter	one
6 Miscella tank	one
5 Pumps	five
4 Percolators	four
3 Platform Balance	one
2 Crusher. Siever	one
1 Dryer	one
Item	Reqd.Nos.

Capacity-100 kg/Batch

Flow diagram-Production of Lityazol Cemil from *Scolymus hispanicus*

Maria B. Narasimha UNIDO
Sedat H. Beis TBAM
Eskisehir
Jan 1, 1994

Specifications of bought out items

1. Steam Boiler

Oil fired or coal fired boiler

Capacity: 500 kg/hour from and at 100^{o}C working pressure 3kg/cm^2. With safety fittings as per govt. regulations.

Analysis of water available at the plant site to be provided by the company management.

If oil fired type, include:

- Bulk fuel oil storage tank with immersion heater at the outlet end.
- Overhead day storage tank with immersion heater
- Fuel oil pump, before preferably geared pump 25 x 25 mm 10 meter total head

2. Centrifugal open impellor type pump. Mono-block 25 mm suction 19 mm delivery. 10 m total head, with flame proof (explosion proof) motor and starter. Non return values. All contact parts SS304-5 hrs.

3. 4 way to & fro, up and down motion electrically operated pully block capacity, 500 kg.

4. Electrically heated shelf dryer. Shelves should be of stainless steel 50 kg crushed root bark per batch. Initial moisture content about 25% final moisture content of about 7%.

Design Drawings

Design calculations for complete plant have been worked out and handed over to the counterpart engineer with detailed instructions.

Shop drawings of individual plant and equipment are being drawn and is expected to be ready in about a week's time. Hence design drawings have not been included in the report.

Annex 8

PERSONS CONTACTED

1. Mr. M. Rasit Karaosmanoglu	Managing Director Dr. Cemil Sener Lab. Manisa
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4. General Manager	Bottle washing M/C Izmir
5. Mr. Güran Oktay	Factory Manager Seker Makina Eskisehir
6. Dr. M.Kemal Hussein	UCD-Ankara
7. Prof.Dr. K.H.C.Baser	Director MPRC-Eskisehir
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Annex 9

Backstopping Officer's Technical Comments based on the work of M.B. Narasimha, SI/TUR/93/801/11-01

The activities carried out by the expert are well presented in the report. The work assigned to the expert has been well performed and the initial activities leading to upgrading the quality and increase of yield of the product have been carried out. The host institution is expected to complete same activities as advised by expert to prepare for the second mission of the expert to complete project activities. Some work will involve active participation of the Medicinal Plants Research Centre (MPRC). The Backstopping Officer agrees with the recommendations of the expert on diversification of products, establishment of a small quality control laboratory and recruitment of the minimum required technical staff and hopes that urgent action will be taken in this regard.