OCCASION

This publication has been made available to the public on the occasion of the 50th anniversary of the United Nations Industrial Development Organisation.

DISCLAIMER

This document has been produced without formal United Nations editing. The designations employed and the presentation of the material in this document do not imply the expression of any opinion whatsoever on the part of the Secretariat of the United Nations Industrial Development Organization (UNIDO) concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries, or its economic system or degree of development. Designations such as "developed", "industrialized" and "developing" are intended for statistical convenience and do not necessarily express a judgment about the stage reached by a particular country or area in the development process. Mention of firm names or commercial products does not constitute an endorsement by UNIDO.

FAIR USE POLICY

Any part of this publication may be quoted and referenced for educational and research purposes without additional permission from UNIDO. However, those who make use of quoting and referencing this publication are requested to follow the Fair Use Policy of giving due credit to UNIDO.

CONTACT

Please contact publications@unido.org for further information concerning UNIDO publications.

For more information about UNIDO, please visit us at www.unido.org

19779

RESTRICTED

DP/ID/SER.A/1590
27 July 1992
ORIGINAL: ENGLISH

INDUSTRIAL UTILIZATION OF MEDICINAL AND AROMATIC PLANT RESOURCES FOR THE PRODUCTION OF PHARMACEUTICALS

DP/TUR/88/001/11-02

TURKEY

Technical report: Phytochemical methods*

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

Based on the work of S. Dev, Phytochemist

Backstopping Officer: T. De Silva, Chemical Industries Branch

United Nations Industrial Development Organization
Vienna

---

* This document has not been edited.

V.92-55968

CONTENTS

1 . INTRODUCTION

The Medicinal Plants Research Centre (Tıbbi Bitkiler Araştırma Merkezi, TBAM), Anadolu University, Eskişehir-Turkey, was set up in 1982. Its facilities and activities were greatly expanded by assistance from UNDP/UNIDO (Projects: DP/TUR/83/003; DP/TUR/88/001) since 1983 and the present buildings and facilities were officially declared open in November, 1986. Currently, a three-storied building is being constructed adjacent to the preseeet facilities and this will greatly augument the activities at the Research Centre.

The Research Centre is reasonably well-equipped to undertake Research and Development activities in the area of extraction and analysis of active principles and essential oils from higher plants.

The present mission (DP/TUR/88/001/11-02) was undertaken on behalf of UNIDO to assist the Director, TBAM in selecting potentially economically valuable medicinal and aromatic plants for R and D work and for imparting training in methods for phytochemical research with special emphasis on terpenes (Annex 1). Unfortunately due to a communication gap, this mission had to be split, as on arrival for the mission in April, it was found that TBAM would be closed for vaccation (Bayrami) from April 12 to April 24. Thus. this mission covers the periods April 4 to April 10, 1991. and Nov. 7 to Nov. 20 1991. Though, an earlier report covering the April visit has already been submitted, this report is a consolidated one covering as well the first leg of the mission. However there are some deletions from the eralier report as because of shortage of time, work could not be initiated ever during November visit. On the other hand, the main purpose of the mission in imparting some training in methods of phytochemical research , and identification of relevant projects for being undertaken at the Research Centre, appear to have been met.

2 ACTIVITIES

2.1. Discussions with the Director TBAM

During discussions, the Director, Dr. K.Hüsnü Can Başer emphasised the importance for essential oils and aroma chemicals industry for local economy especially in view of the increasing demands for perfumes and flavours for various consumer goods. At present, most of these requirements are being met by imports, while on the other hand relatively large quantities of certain valuable raw materials for aroma industry are being exported (Annex 2). However, it should also be noted, that some essential oils are being produced in Turkey. (Annex 3) and that its production of rose oil is almost 50 % of the world production.

Keeping in view the above discussion and the job description (Annex 1), the laboratory work was organised with emphain on aromatic plants and aroma chemicals especially those derived from terpenes.

2.2. Laboratory Activity

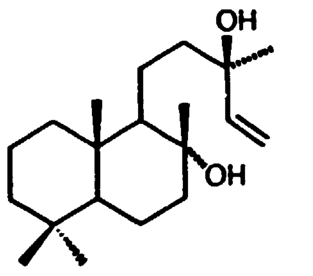
A number of projects were initiated in an effort to expose the worker scientists (Annex 4) to different laboratory techniques and methods of value in phytochemistry and further processing of phytochemicals. All projects were based on Turkish raw materials, and the target was a product which could later have an economic impact, either as an item of export or as an import substitute.

2.2.1. Sclareol Sclareol, $C_{20}H_{36}O_2$ (m.p. 105.5-106°) is a diterpene first isolated[1] in 1928 from the leaves of *Salvia sclarea* (clary sage). It has also been isolated for the higher boiling fractions of the essentiol oil of clary sage[2]. It occurs in the clary sage concrete (hexane extract of the plant malerial) to the extent of 50-60 %[3]. It forms the starting material for thie commercial production of an important aroma chemical [Trade name Ambrox (Firmenich), Ambroxane (Henkel)] with ambergris fragrance (cf. 2.2.2.).

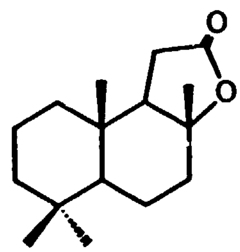
Since clary sage is cultivated in Turkey and the herb is exported (Annex 2), it appeared worthwhile to focus attention on it, especially, as both the essential oil and the sclareol which can be obtained from the spent herb are commercially important. World production of clary sage oil is around 45 tonnes[3] and the chief producer is USSR, though small quantities are still produced in France[4]. The oil has use both as a fragrance and flavouring malerial. The oil yield is ~ 1% and the oil is rather expensive[5]. The oil produced earlier at TBAM was of internaitonal quality. Thus the national essential oil should be commercially produced in Turkey and the marc can be used for extraction of sclareol. During this Mission, work was carried out with spent plant material (floweving tops and overground foliage) available from the earlier distillation experiments.

Extraction of the material was studied with different solvents using Soxhlet apparatus. All extracts were dark green and the yields were : hexane (3 %), ethyl alcohol (12%), acetone (7%). All extracts contained significant amounts of sclareol (TLC : 1% methanol is Chloroform as solvent ; Silica gel plates ; spray, 0.5 % vanillin in 50 % phosphoric acid aq. to give purple spot), though the hexane extract was richest. Hence marc was extracted with hexane, the solvent removed and the residue treated with acetonitrile and the solid (crude sclareol) collected and purified as usual.

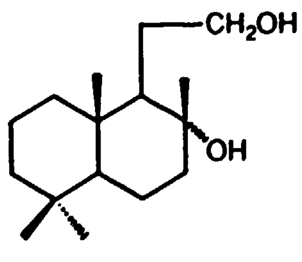
2.2.2. Sclareol to Ambrox There are several routes to Ambrox (4)[6]. Commercialy it is produced from sclareol, using oxidation of sclareol (1) to sclareolide (2) descrined by Ruzicka and Janot in 1931[9]. (±)-Ambrox has also been synthesised by totally synthetic methods[7]. However, (-)-Ambrox (4) which is obtained by degradation of (-)-sclareol is far superior and fetches a price of US.$ 1000/kg[7]. It is imported into Turkey, though the exact figures could not be obtained.



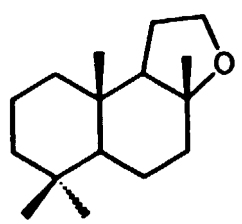
(-)-Sclareol
1



Sclareolide
2



3



(-)-Ambrox
4

Some sclareol and sclareolide were available in the TBAM laboratory, so along with the isolation work on sclareol, work on the preparation of Ambrox could also be undertaken.

A single experiment for the oxidation of sclareol (5.0 g) to sclareolide as per Ruzicka's procedure[2] was carried out (sclareol 5 g in 50 ml AcOH + 130 ml of oxidizing solution prepared from 22 g comm. CrO_3+30 ml water + 200 ml AcOH). Reaction was carried out at 22-27° for 24 hr and at ~ 50° for 24 hr.) under TLC monitoring (Solvent : 1 % MeOH in $CHCl_3$) and finally worked up as per the literature procedure.

A single experiment for lithium aluminium hydride reduction of sclareolide (2, 2.5 g) in THF (25 ml containing 0.38 g LAH as suspension) was carried out first at room temperature (~20°, 1/2 hr) and later at reflux (1 hr) and worked up in the usual manner (EtOAc, followed by 5 % H_2SO_4 aq.). Product vas extracted with ether and furtherprocessed in the usual manner to get 2.4 g of diol 3 (TLC pure, m.p. 122-125°). An IR spectrum was recorded.

Cyclization of diol (o.5 g) with p-TSA (10 mg) in toluene (10 ml)was carried out by distilling off half of the toluene from the mixture. TLC showed excellent conversion (some isoAmbrox was also formed).

2.2.3. α-Terpineol Several *Pinus* spp. grow in Turkey, the commercially important ones are *P. brutia* and *P. sylvestris*. Turpentine is obtained by gum-resin distillation,solvent extraction of pine stumps and as a byproduct (Sulphate turpentine) in kraft pulping. Production figures are not available though in 1984 some 554 tonnes[8] of gum turpentine was produced. At present, it appears more of sulphate turpentine is available.

Turpentine is the traditional source of aroma chemicals based on pinene chemistry[9]. It was decided to demonstrate preparation of α-terpineol and camphene from α-pinene.

A sample of 97 % pure α-pinene from solvent extracted turpentine was available. This was used for both conversiones.

Several methods are available[10] for the production of α-terpeneol from α-pinene. However a process described recently utilizes H_3PO_4 aq. and is economical and convenient to use. The scientist concerned was introdued to this method and several experiments were carried out to arrive at the best conditions. The best experiment was conducted at ~ 22° (α-pinen 25 ml, 50 % phosphoric acid aq. 25 ml Tween 80 0.5 ml) with stirring (magnetic). After 7 hrs all the pinene had been consumed to give 43 % α-terpineol (GLC). This work will have to be pursued to arrive at the optimal conditions. Lower temperature (~ 15°) proved beneficial. The main advantage of this method is that the aq. phosphoric acid, after proper adjustment can be reused any number of times.

2.2.4. Camphene Camphene is an important material for several perfumery compounds[9]. Commercially, camphene is produced by isomerisation of pinenes on a titanium catalyst, usually m-titanic acid[11].

A procedure was worked out for the preparation of m-titanic acid (from titanium dioxide and NaOH aq.) along usual lines. A method was also evolved for testing the activity of the catalyst. The catalyst was next used for the isomerization of α-pinene (100 ml α-pinene, 250 mg catalyst ; 135° refluxing under reduced pressure, with any water entrainment).

2.2.5. Oakmoss absolute This is possibly the most important of all products derived from oakmoss . True oakmoss is the lichen *Evernia prunastri*. This material is available in Turkey and is exported along with other lichens. A small sample of this material, which had been collected in 1986, was available and it was considered worthwhile to prepare its absolute.

The lichen (50 g) was carefully shredded and hot extracted with purified hexane to finally yield 1.7 g of concrete as a rather solid green mass. The usual average yield reported[12] is 2-4 %. This product was next converted into its absolute (yield 50 % of concerete) by the usual procedure of extraction with ethyl alcohol.

Thus, the preparation of oackmoss absolute from the Turkish raw material appears to be satisfactory in term of the yield and quality. However, more work must be carried out by large scale extraction and evaluation of the product by good perfumers and possible buyers. Characterization of the product in term of its chemical contituents will also be essential, a good referance on the subject is a publication by Terajima et al.[13]

An industrial continuous process for oakmoss extraction has been described.[14] The current selling price is around £ 100 /kg.[5]

2.2.6. Styrax resinoid and styrax absolute Styrax is a natural balsam formed on injury to the styrax tree (*Liquidambar orientalis* , *L.styraciflua*)15. *L.orientalis* grows in Turkey and its balsam is exported (Annex 2).

Styrax is a viscous semi-solid mass of brownish gray colour having a water content of up to 30 %, which usually shows up on the surface.

A simple procedure has been developed to get either the resinoid or the absolute. The crude styrax is mixed with up to one and a half of its weight of plaster of Paris (dehydrated gypsum) or anhydrous sodium sulphate to get a workable material, which is charged in an extractor, suitable for hot extraction. With alcohol as the solvent, the absolute was obtained as brownish transluscent.viscous mass. On the other hand, toluene furnished a lighter coloured, more transparent product (resinoid). Both the products had good odour impact. An essense (colourless soln. of the odourous principles in the solvent)was also prepared by co-distilling the resinoid with propylene glycol. This appears to be an innovative product of good value for flavonning purposes. The current market price[5] of styrax absolute is around £ 52/kg.

2.2.7. General remarks Work described in this section of the report is of the most preliminary character. If development of any or all of these products is envisaged, additional laboratory work to work out the optimum condions will be necessary. Special attention will have to be paid to energy aspects. This has been stressed to the workers who participated in the projects.

2.3. Lectures

A total of five lectures were delivered. The first three were designed to emphasize the economic potential of plant wealth, and the role chemistry can play in achieving end results. The fourth lecture concerned designing of aroma molecules. The last was an informal lecture-cum-tutorial on aspects of organic chemical laboratory practice.

2.3.1. Higher Plants and Chemical Industry (April 9., 1991) This lecture placed in proper perspective the economic impact of higher plants in chemical economy.

2.3.2. Drugs from higher plants (April 10) After reviewing the importance of higher plants in modern medicine, work from author's own laboratory was presented.

2.3.3. Restructuring Molecules of Nature (April 11) This lecture was designed to stress how chemistry can convert apparently non-useful secondary metabolites from higher plants into economically important compounds.

2.3.4. Molecular Engineering for Sandal Aroma (Nov.13) This lecture brought out that compounds can be designed with targeted application, taking, the example of structure and sandalwood aroma.

2.3.5. Aspects of Organic Laboratory Practice (Nov.19) It was felt desirable that I advise the scientists at TBAM on various aspects of working in an organic chemical laboratory, including the saftey aspects. This lecture -cum-tutorial was to that effect.

3.CONCLUSIONS AND RECOMENDATIONS

1. Essential oils, aroma chemicals, food colours and related products are important inputs for a variety of food preparations and consumer goods. Though, Turkey has a well-developed rose oil industry, there is a distinct need to expand the base, making use of indigenous raw materials.

2. TBAM Research Centre is well-suited to undertake this responsibility as a certain amount of infrastructure for this type of work exists, though aditional equipment and manpower will have to be inducted. The centre has already undertaken expansion of work space by building additional floor area.

3. Since production of value-added products from a given raw material is essentially achieved by the application of chemical transformations, it will be essential to strength on the chemistry base at TBAM, which is practically non-existent at present. There is a distinct need to induct.

(a) Manpower (Chemistry graduates and some with post-graduate/doctorate attainments)

(b) Equipment (glass reactors for the laboratory and pilot plant, for catalytic hydrogenation, high pressure reactions, catalytic vapour phase reactors, etc.).

It will also be necessary to furnish the library with books and journals dealing with chemistry.

4. Following projects/problems appear to deserve attention in the first phase. These projects should have good economic potential.

(a) *Rose oil* Though Turkey is one of the main producers of rose oil, rose concrete and rose absolute, the scientific base of this industry will have to be strengthened. New methods of preparing the absolute from the concrete are on the horizon or are even being used (e.g. molecular distillation, carbondioxide extraction). Microbiological treatment of the waste of the rose concrete has led to additional quantities of rose oil.[3]

(b) *Clary sage* This material is strongly recommended for development as both the oil and the byproduct sclareol are commercially valuable. Work should be undertaken in collaboration with some agricultural university/college to work out agronomic parameters for commercial cultivation of the herb.

(c) *Turpentine* Turkish turpentine (*ex Pinus brutia , P.sylvestris*) is rich in α-pinene, which is the starting point for the manufacture of a variety of aroma chemicals. Fractionation data should be collected and a commercial party encouraged to put up a fractionation unit for the productine of α-pinene. Work should be started simultaneously on the transformation of α-pinene. Some work was initiated during this Mission. Other items worth pursuing are campholenic aldehyde-based sandalwood aroma compounds[16]. besides camphene-derived products such as isbornyl acetate and camphor.

(d) *Linalool* There are several local plants of the genus *Origanum* which are rich in linalool[17]. These should be developed, again in colloboration with an agricultural university, as a commercial source of linalool.

(e) *Absolutes* As already pointed out Oakmoss and Styrax absoiutes are obvious choices. Turkey is also famous for its tobacco. Tobacco leaf absolute[18] is another item worth exploring.

It should be emphasised here that for each item developed, proper specifications will have to be laid down. The saftey aspect, that is the pesticide residues and heavy metal contamination will have to be kept in mind in the case of resinoids, absolutes and essential oils.

REFERENCES

1. Y.Vomar and A Jermstad, *Compt. rend.* 1928, **186**, 517,783.
2. L.Ruzicka and M.M.Janot, *Helv. Chim. Acta* 1931, **14**, 645.
3. M.Petrzilka and C.Ehret in *Perfumes : Art, Science and Technology* (Edits : P.M.Müller and D.Lamparsky) pp 499-531. Elsevier Applied Science, London (1991).
4. International Trade Centre UNCTAD / GATT (Geneva, 1986) : *Essential Oils and Oleoresins : A Study of Selected Producers and Major Markets.*
5. *Cosmetic World News* 138-139 (1991) : Clary Sage (Russian) £ 95.00/kg
6. G.Ohloff in *Fragrance Chemistry* (Ed. E.T.Theimer), p 535. Academic Press, New York (1982).
7. G.Frater and D.Lamparsky in *Perfumes : Art Science and Technology* (Edits : P.M.Müller and D.Lamparsky) p 547. Elsevier Applied Science, London (1991).
8. Bijen Erkut, M.S. Thesis (Ege University), İzmir, 1986, p 12.
9. See e.g.: *The H&R Book Guide to Fragrance Ingredients*, p 23. Johnson, London (1985).
10. See e.g. : P.Z.Bedoukian, *Perfumery and Flavoring Synthetics* p 393. Allured Publishing, Wheaton (1986).
11. See e.g. : G.Etzel, US Patent 2551795 (1951).
12. Ref. 9, p 106.
13. Y.Terazima, H.Ichikawa, K.Tokuda and S.Nakamura in *Flavors and Fragrances : A World Perspective* (Edts. : B.M.Lawrence, B.D.Mookerjee and B.J.Wills) p 685. Elsevier, Amsterdam (1988).
14. J.-Bats, J.-J.Moulines, A.-M.Lamidey, D.Coutiere and J.-F.Arnaudo, *Perfumer and Flavorist* 1990, **15**, (6)15.
15. S.Arctander, *Perfume and Flavor Materials of Natural Origin*, pp 597-601. Elizabeth, N.J., USA (1960).
16. Ref. 7, p 562.
17. Personal Communication from Prof. Dr. K.Hüsnü Can Başer.
18. Ref. 5, p 129 ; Ref. 15, p 618.

UNITED NATIONS INDUSTRIAL DEVELOPMENT ORGANIZATION

JOB DESCRIPTION

DP/TUR/88/001/11-02

Post title	Phytochemist
Duration	0.5 w/m
Date required	November 1991
Duty station	Eskisehir (Turkey)
Purpose of project	To develop the industrial utilization of medicinal and aromatic plant resources for the production of pharmaceuticals.

Duties

The expert is expected to work in the Medicinal Plants Research Centre, University of Anatolia. The expert will be specifically required to:

1. Assist the Director, Anadolu University Medicinal Plants Research Centre, in programming training in phytochemical methods and in selecting potential medicinal and aromatic plants for research and development.

2. Give advice to the Director and national counterparts on use of modern analytical methods, phytochemical research techniques, for quality assessment of raw materials and products, and in terpene chemistry.

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

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

Project Personnel Recruitment Section, Industrial Operations Division

Qualifications	Phytochemist with post doctoral experience and experience in developing countries.
Language	English/Turkish
Background information	The Government is interested in developing the country's considerable natural resources of medicinal and aromatic plants. Two specialist missions have previously stressed the need to build an industry which could process such plants with pharmaceutical products, both for local use as well as for export. The Medicinal Plants Research Centre is part of the University of Anatolia, the associated facilities of which are also available to the project. The Government expects the Centre to develop as an R + D Centre to serve an industry which will provide the country with plant-derived pharmaceuticals. The Centre will at first be required to generate the scientific know-how and process technology on which such an industry may be based.

Annex 2

Exports of Aroma Raw Materials from Turkey*

No	Product	Quantity (tonnes)
1	Origanum (leaves)	3200
2	Lichen (two varieties)	24
3	Aniseed (seeds)	2600
4	Coriander (seeds)	87
5	Cumin (seeds)	8900
6	Clary Sage (herb)	560
7	Horseradish (root)	354
8	Styrax	13

* Prime Minister's State Statistics Institute (Centre for Export Promotion), Turkey; Cited in Tıbbi ve Aromatik Bitkiler Bulletin, May 1991.

Annex 3

Essential Oil Export from Turkey*
(1990)

No	Product	Quantity (tonnes)
1	Rose Oil	8.5
2	Origanum Oil	2.5
3	Other Essential Oils	1.4

* Same source as Annex 2.

Annex 4

Names and scientific qualifications of personnel who participated in the laboratory investigations

1. Sedat Hakkı Beis, Chemical Engineer,M.S.
   (Sclareol, Styrax)
2. Temel Özek, Chemical Engineer, M.S.
   (Terpineol, Camphene)
3. Zeynep Topraklı, Pharmacist, B.S.
   (Sclareolide)
4. Müberra Koşar, Pharmacist, B.S.
   (Oakmoss absolute)
5. Hülya Tanrıverdi, Pharmacist, M.S.
   (Ambrox)
6. Mine Kürkçüoğlu, Chemical Engineer, B.S.
   (Gas-Liquid Chromatography)

Annex 5

**Backstopping Officer's Technical Comments
based on the work of Mr. Sukh Dev
DP/TUR/88/001/11-02**

The consultant has written a consolidated report incorporating the work carried out during his two split missions. The report clearly presents the important and useful work initiated by him and very relevant recommendations in identifying the prospective areas of research.

He has trained the counterpart staff in research methods and enhanced the knowledge of counterpart staff by conducting lectures in the field of natural products chemistry as applied to drugs and aroma chemicals.

The need to strengthen the chemical laboratories to carry out large scale reactions and the necessity to recruit chemists as recommended by the consultant should receive the attention of the Institute as priorities in establishing a research base.