Assessing the Technical and Economic Viability of the Ethanolic Extraction of Artemisia Annua

A study commissioned through
Medicines for Malaria Venture (MMV)

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Introduction

Artemisinin based combination therapies (ACTs) could reverse the still ongoing upsurge of malaria in Africa and elsewhere. ACTs are able to eliminate malaria parasites that have become resistant to the remedies that have been previously in use. Currently, not enough of these ACTs are produced and their production costs are too high, so most of the people who would need them have no access. The expensive element in the ACTs is the artemisinin which is extracted from the plant *artemisia annua*. Bringing down the costs of extraction is one way to contribute towards a solution to this problem. The extraction technology chosen should, however, also be as safe as possible.

As far as a can be concluded from a survey of the relevant literature, almost all commercial extraction of *artemisia annua* is currently done with solvents that pose considerable dangers to occupational health and to the environment. The solvents that have so far been successfully applied, mainly in Vietnam and China, are diethyl ether, petroleum ether, benzene, acetone, toluene, acetonitrile and dicholoromethane , all of which are very poisonous and dangerous, as well as n-hexane, which also needs to be handled very carefully for safety reasons.

N-hexane is the most commonly used solvent. While the risks associated with it can – in principle - be controlled by suitable technology and very careful monitoring, small leaks can never entirely be ruled out. Moreover, the disposal of large quantities of spent leaves that might be contaminated, as well as contaminated liquid from cleaning operations, require careful containment operations. Furthermore, facilities working with n-hexane are barred by EU regulations from extracting a number of food additives, which could otherwise offer interesting markets for diversification. It must also be noted that industrial accidents with n-hexane, which is quite explosive, do occur regularly, even in industrialised countries. Moreover, extraction rates achieved with hexane, usually around 70%-75%, are not as high as one would like them to be. After purification the yield is reported to be only 40% to 65% of the artemisinin present in leaves [1].

The question is, whether there are other, more effective, alternatives which are also more environmentally friendly.

Publicly available information that would help to answer this question is difficult to find. Published empirical studies on extraction technologies relating to *artemisia annua* are scarce and usually restricted to small lab-scale experiments, which may not be relevant to larger scale operations. Private entrepreneurs rarely add to the general pool of knowledge, they are not necessarily looking for environmentally optimal solutions and do not publish their results. While it would be desirable that the knowledge regarding efficient extraction procedures is accessible to all in the field of extracting *artemisia*, the usual practice in the field of commercial extraction is that each company works out its own extraction protocol – often with very high licence fees paid if specialised companies assist in developing such a protocol. After having incurred these costs companies hold the results secret. While this is an understandable reaction to the

[1] Information from Charles Giblain, Bionexx, Madagascar

market economy, it does mean that there is a danger that the work is repeated again and again. It also makes the end product more costly than necessary.

Examining potentially promising alternatives to the extraction with hexane, MMV has recently released a study looking into the potential of some novel solvents and extraction technologies[2]. In this study, the potential of using ethanol was only mentioned in passing.

The study presented here aims at looking more carefully at the technical and economic viability of ethanolic extraction of *artemisia annua*.

In order to asses the potential of ethanolic extraction, a survey of the relevant literature, consultations with experts, laboratory scale experiments as well as extraction trials on a somewhat larger scale were conducted.

In order to assess the results in economic terms, quotations from suppliers of the extraction equipment as well as the intermediate goods were collected. Other capital and running costs (buildings and wages for instance) were assessed by local experts, taking Tanzania as an example.

Extraction with Ethanol: General Considerations

Possible Advantages

Among the different options for extracting artemisia, the extraction with ethanol, if economically feasible, could offer certain advantages over hexane.

1. Higher extraction rates

Most studies that compare extraction technologies state that ethanol is a more effective solvent and that extraction rates that can be achieved with ethanol are higher than those achieved with hexane. Whether and to what extent this assertion is true will be examined in this study.

2. Local availability and lower price of the solvent

Hexane is a byproduct of petroleum refining. In those African countries, which do not have a refinery, it has to be imported. Many African countries already produce ethanol, usually as a sideline to sugar production and many countries which do not yet produce ethanol are planning to do so in the near future. In terms of costs the difference between the two solvents is not large: The price of ethanol (96% purity) is rather volatile, ranging between 0.39 cents and 1.30 USD per litre depending on the time of purchase and the place of origin, but it is somewhat lower than the price for hexane which appears to fluctuate between 0.63 and 2.00 USD per litre depending on time and place [3]. Since during most of the extraction, recycled rather than fresh solvent is used, the original price of the solvent is, however, of less importance than the quantity of solvent that is required to perform the extraction and the costs of the recycling of this solvent.

[2] Cutler Malcolm, Rifkin Alexei; Comparative Assessment of Technologies for Extraction of Artemisinin, A summary of report commissioned through Malaria Medicines Ventures (MMV), August 2006

[3] own observations from internet postings and correspondence with companies.

3. Better occupational and environmental safety

Hexane is more often involved in industrial accidents and is more dangerous to human health and the environment than ethanol, so on these grounds a shift from hexane to ethanol would be desirable. (For further explanations of the differences regarding hazards see Annex 1)

4. More flexibility for the production of other phytopharmaceutical and nutraceutical products

Ethanol can be used to extract many different botanicals and would allow the utilisation of African herbs and spices for medical and nutraceutical purposes.

Propane, butane, carbon dioxide, ethyl acetate and ethanol are the only solvents allowed in the production of food additives in the EU without any restriction. For 1,1,1,2-tetrafluoroethane (hydrofluorocarbon R134a) the EU has proposed a limit of 20.2 mg/kg that is allowed in flavouring additives, for hexane the limit is 1 mg/kg [4]. Traces of hexane are not allowed in various health products. An extraction facility based on ethanol would widen the choice of products that could be produced for medicinal purposes. While hexane is the most common solvent in the field of edible oil extraction and for the extraction of natural pesticides, extracts of herbal medicines and food additives are usually produced with water, ethanol or carbon dioxide. The most common herbal products in Europe are extracts from chamomile, mint, echinacea, valerian, gingko and St. Johns Wood, but potentially there are many more that could be produced for a growing market for "natural medicines" in Europe and in Africa.

Arguments against extraction with ethanol

1. The poorer selectivity of ethanol

The main argument against the use of ethanol, which is repeated in various publications comparing the different solvents, is its poor selectivity[5, 6, 7]: It is claimed that while ethanol may be more efficient in extracting artemisinin from the plant than hexane, it co-extracts more contaminants, which may require more expensive purification and/or lead to a loss of most the additional artemisinin that has been gained. Since the nature of these co-extracted substances and the problems associated with removing them are, however, never explained, there is no convincing proof that purification needs to be more complicated, more expensive or less successful. This matter has to be settled by finding an appropriate purification protocol for the ethanolic extract once the extraction conditions have been worked out.

2. The fact that ethanol forms an azeotrope with water

Extraction with ethanol may entail higher capital and running costs due to the need to remove accumulated plant liquid from the solvent. Ethanol mixes with water and forms an azeotrope which cannot be separated during the evaporation of the solvent. Since the dried leaves contain some liquid (8 – 10% under good drying conditions), this liquid mixes with the ethanol and accumulates until the solvent requires rectification. The

[4] See EUR-Lex - 52003PC0467 - EN

[5] Dixon, Thomas; Report into the feasibility of Production of Artemisia annua in Tanzania and Kenya And Extraction of Artemisinin in Tanzania and/or Kenya, October 5, 2004

[6] Heemskerk, Willem, Schallig Henk,de Steenhuijsen Piters, Bart; The World of Artemisia in 44 Questions, The Royal Tropical Institute (KIT), March 2006

[7] Haynes, Richard K. : "From Artemisinin to New Artemisinin Antimalarials: Biosynthesis, Extraction, Old and New Derivatives, Stereochemistry and Medicinal Chemistry Requirements" Current Topics in Medicinal Chemistry, Volume 6, Number 5, March 2006, pp. 509-537(29)

equipment for rectification has to be added to the capital costs. While this addition (about 7 – 8% of total capital costs) would be bearable, the question is, how high are the running costs (with steam) of the rectification process? These running costs depend on the degree to which the solvent is diluted during the extraction process. This problem will be examined in the study presented here.

3. Lower stability of artemisinin in ethanol

There is one publication which suggests that artemisinin is not sufficiently stable in ethanol[8]. This point will also be addressed.

4. Poor solubility of artemisinin extracted with ethanol

Artemisinin that has been dissolved in ethanol forms crystals, which are smaller and "less soluble in water" than artemisinin crystallised from hexane[9]. Less soluble artemisinin crystals may result in higher derivatisation costs when the artemisinin is converted to dihydroartemisinin. Whether data on the solubility of artemisinin crystals harvested from aqueous ethanol have any relevance to this issue is, however questionable. First of all, the problem may have been due to the dilution of the solvent rather than the ethanol. Secondly, solubility in water is not relevant since artemisinin is hardly soluble in water anyway. It would only matter if poor solubility occurs in methanol, which is often employed in the first solubilisation step of the derivatisation process. Thirdly, extracts are usually recrystallised during purification. A final recrystallisation with ethanol is common with hexane extracts, while with ethanolic extracts, a final recrystalllisation is usually performed with ethyl-acetate/hexane.

5. Problems with ethanol due to the fact that in diluted form it is used in beverages

Since ethanol is also included in alcoholic beverages, which may in some countries entail special taxes, it is necessary to distinguish the solvent clearly as an industrial alcohol. This is normally done by spiking the solvent with ethyl acetate, isopropanol or methanol which make the alcohol unpalatable . This addition is designed to protect the product from abuse. Such mixtures are available in Africa and carry no special tax. It is suggested that for the purpose of artemisia extraction an addition of ethyl acetate would be preferable, as it enhances the extraction power of the solvent.

[8] Daniel L. Klayman, AI J. Lin, Nancy Acton, John P. Scovill, James M. Hoch, Wilbur K. Milhous, Anthony D. Theoharides, Arthur S. Dobek: "Isolation of Artemisinin (Qinghaosu) from Artemisia annua growing in the United States", Journal of Natural products, Vol 47, No 4, pp 715-717, Jul-Aug 1984 .

[9] Cutler and Rifkin, op. cit. p. 17

Summary of General Considerations and Questions for the Research

From the considerations outlined above, it is clear that the empirical investigation into the potential of using ethanol as a solvent for the extraction of *artemisia annua* would have to answer the following questions:
1. Is ethanol really much more efficient as a solvent than hexane and is it better to use diluted or relatively pure ethanol ? (This question was addressed in small scale trials).
2. Is artemisinin unstable in ethanol or not? (This question was addressed in the small scale trials).
3. How much more impurities – in terms of weight – are co-extracted with ethanol compared to hexane and what can be said about the nature of these impurities. (Answers to this were sought in the small scale trials).
4. What is the optimum extraction time and the best ratio of solvent to leaves in the extraction ? (This issue was pursued both in small and in larger scale trials).
5. What is the optimum design for the extractor. (This question was addressed in the larger scale trials).
6. Is it necessary to heat the solvent or not? (The larger scale trials were used to answer this question).
7. Can the amount of solvent used be reduced by a suitable extraction protocol. (This question was addressed in the larger scale trials)
8. How much additional costs have to be incurred because of the need for rectification? (This question was answered from the larger scale trials once an optimal extraction protocol had been established)
9. How can ethanolic extracts be purified ? (Since the scope of the study did not include purification, answers were mainly sought in the available literature and a few preliminary experiments.)
10. Is extraction with ethanol economically viable? (An estimate was attempted based on the data from the larger scale trials) .

Experimental Section: Trials with Ethanolic extraction

Problems of Measurements

The Extraction Rate or Extraction Efficiency is measured as the percentage of the original quantity of artemisinin available in the fresh leaves that can be detected in the extract after extraction.

The few trials on ethanolic extraction that have been reported in the literature do not discuss problems in measuring this outcome, although such problems do exist.

1. Determining the artemisinin content of leaves and extracts

There are a number of different methods for determining artemisinin content of leaves and extracts and different laboratories use different methods[10]. Apparently, several methods can lead to reliable results, although most experts currently prefer HPLC-ELSD for precise measurements. In this case it is, however, very important that the solid-liquid extraction of the material that is subjected to HPLC is performed in an appropriate fashion. During the current trials, five different laboratories were

[10] For an overview see: P. Christen* and J.-L. Veuthey: "New Trends in Extraction, Identification and Quantification of Artemisinin and its Derivatives" Current Medicinal Chemistry 2001, 8, 1827-1839 1827

approached, of which only one, after some adjustments produced reliable data[11]. The most reliable method appeared to be the following:

Testing of leaf content: 20g of plant material was refluxed with 150ml of methanol for 4 hours. The solvent was filtered off on a sintered funnel and the plant material was extracted further three times each with 50ml of MeOH. The extracts were combined and the solvent removed on a rotary evaporator.
The above process was repeated once more on the residual plant material.

Over 90% artemisinin was extracted in the first extract. The second extraction yielded an additional 20% extract by weight (compared to the first extract). The concentration of artemisinin in the second extract was less than 5% in comparison to that in the first extract.

HPLC analysis of ethanolic extracts:
The ethanolic extracts were evaporated under reduced pressure or dried under a stream of nitrogen and re-dissolved in a known volume of HPLC solvent. The solution was filtered (or centrifuged) and analysed directly by HPLC.

HPLC conditions were as follows:
The HPLC system was supplied by Gilson and the ELSD detector by Polymer Laboratories (model PL-EMD 960). The latter was operated at 55 deg C with a nitrogen flow rate of 3.31 ml per min. A Synergy 4micron Max RP column of 150 x 4.6 mm (manufactured by Phenomenex) dimensions was used and eluted with a mixture of acetonitrile (5parts): methanol (2 parts): water (3parts) at a flow rate of 0.5 ml per min. The injection volume was 50 microlitres and retention time for artemisinin is 9.8min.

All analyses were based on reference graphs derived using pure artemisinin. A minimum of 2 replicates were carried out for each sample

General notes:
Based on many experiments, the ELSD method for detection was considered to be superior for the analysis of artemisinin content when compared with methods based on UV detection (especially for plant extracts) and hydrolysis of artemisinin.
Artemisinin standard: An industrial sample of artemisinin was recrystallised four times.
The concentration of the sample of artemisinin was tested by ELSD.

2. Estimating the artemisinin content of the plant material

When determining the original artemisinin content of the leaf material , it is important to remember that hybrid *artemisia annua* plants are very heterogeneous in terms of artemisinin content. Not only does the content vary from location to location and according to different cultivation practices, but even plants within the same field will differ in artemisinin content and within the plant itself, the lower part of the plant may contain much less artemisinin than the upper part. Furthermore, during storage the plants at the outside of the bags tend to loose more artemisinin than those stored on the inside. In order to overcome this heterogeneity, the plant material has to be homogenised (thoroughly mixed) before samples are taken. Ideally, a homogeniser (a kind of centrifuge) should be available to do this job. Usually , however, mixing is done by hand and there is always the danger that a small sample taken to determine the artemisinin content of the fresh leaves is not representative of all the material that is used for the trial or, – when trials are conducted on small batches of leaves -, individual

[11] Our method of checking was to monitor the sums of artemisinin of the fresh leaves, the extracts and the spent leaves and in particular the sum that appeared when extraction was prolonged so much that it could be expected that all the artemisinin must have been extracted. This would, however, not have been enough to protect from systematic under- or overestimates if the same factor was applicable to both the leaf analysis and the analysis of liquid extracts. It is, however, possible to detect systematic underestimates if, after extraction and purification, a larger quantity of artemisinin actually turns up than should have been there according to leaf analysis. Initial overestimation is more difficult to detect since the fact that less than the expected quantity of crystals is harvested may be the result of the purification methods.

batches may differ in their artemisinin content from other batches so that the results of the extraction cannot be compared reliably. Care is therefore required to produce samples of leaves that do have the same artemisinin content, both for the testing of the leaves and for the extraction trials.

3. Estimating the quantity of extract that remains trapped on leaves

After the extraction is completed, a certain amount of liquid extract remains on the leaves. Part of this can be recovered by pressing. During extraction trials small hand presses are used whose efficiency varies depending on whether one or two persons are available to operate the press and on the strength of the persons handling the press. As a result, the amount of extract recovered varies and so does the extraction rate. In order to overcome this problem it was decided to use two measurements for the extraction rate: the rate that would have been achieved if all the solvent used would have reappeared in the extract ("total extraction rate" (TR)) and the rate that would have emerged if all batches of spent leaves had been pressed to the same amount of dryness, leaving on the spent leaves not more than 0.40 litres of extract per kg of fresh leaves employed ("standardised extraction rate"(SR).

4. Estimating the quantity of extract that can be harvested

Under experimental conditions where the filling of the extractor with solvent, the transfer of the extract to the container and the filtering of the extract are done by hand, an amount of ethanol which is difficult to quantify, escapes by spontaneous evaporation. On the other hand, the volume of the liquid that emerges as extract will be slightly larger than the amount of solvent employed, because of the extraction of some of the plant juice. For practical purposes it seemed realistic to assume that these two effects would cancel each other out, so that the amount of solvent entering the extraction cycle would be a good estimate for the total amount of extract involved that might in the end either be harvested or remain entrained on the leaves.

Small scale trials: Trials with the Timatic extractor

On the advice of an expert[12] who has been performing trial extractions of *artemisia annua* in Uganda, a small Timatic extractor from Tecnolab (Perugia, Italy) was initially used for the first trial extractions knowing that a larger extractor from Timatic (400 litre capacity) might be used in the extraction facility. The fact that these extractors are inexpensive and that company officials were very co-operative also assisted in the choice of extractor.

[12] Mr. Rodeyns Nicolai of Nalweyo Seed Company (NASCO Ltd)

The Timatic – micro extractor

The Timatic is a solid-liquid extractor that is being used for industrial production of herbal extracts. According to the manufacturer, percolation is achieved by alternating a dynamic phase during which a pre-set pressure is being generated, followed by a static phase so that the solvent is pushed into the plant cells and released again. The pressure phase prevents the formation of channels as well as partial over-saturation of the solvent. Another advantage of this equipment is that it can be used for many different liquid solvents. It is semi-automatic, easy to handle, with a well designed display, with an automatic warning system and an automatic cleaning program.

First set of trials

As will be noted in this and the following trials, the Timatic extractor has to be filled completely with the solvent before the pressure can be built up as required, so a one-litre facility will have to be filled up with a small quantity of additional solvent to fill the pipes that lead to the extraction vessel. On the small extractors there is always some variation from batch to batch with regard to the quantity of solvent used and this variation has to be taken into account in calculation of the total extraction rate.

According to the trial plan, the ethanolic extracts were supposed to be evaporated immediately under low temperature and the tests on the artemisinin content of the extract were to be done on the solid raw extract. The reason for this approach was concern that if the artemisinin stayed in the ethanol for too long before testing, some of the artemisinin could degrade. It took, however, several weeks from extraction to evaporation, six weeks until the spent leaves were analysed for their artemisinin content and about eight weeks from the date of extraction until the extracts were analysed.

During these first trials the analysis of the extracts was done by ILIS (Biel, Switzerland) who described their method of analysis as " HPLC/DAD post column derivatisation (not validated)". The leaf analysis was done by Mediplant (Conthey, Valais, Switzerland) using TLC methods. In all later trials, HPLC-ELSD was used.

1 st and 2 nd trial with Timatic extractors: process data

No of Test	TO 175	and	TO 176		
Equipment:	Timatic Micro 1.0 Litre		filter bag	100 micron	
settings:	Compression	4 Mins	Decompressio	4 Mins	
	ambient temperature		20°C	pressure	8.15 bar
TO 175	duration	5 hrs	(38 Cycles)	Percolations	M0 1-5
TO 176	duration	8 hrs	(60 cycles)	Percolations	M0 2-11
leaves:	Artemisia annua	dry, 8% humidity	not milled, particle size 2 - 6 mm	300 g each batch	

Results of trials

No of test	TO175	TO176
process time minutes	304 (5 hrs)	480 (8 hrs)
quant leaves g	300	300
art content of leaves	0.74%	0.74%
g of art available in 300 g leaves	2.22	2.22
solvent used	ethanol 100 %	ethanol 100%
quantity of solvent used ml	1050	1050
tot solvent recovered ml	950	950
g of art per 500 ml of liquid extract	0.64	0.98
g of art per 1 ml of liquid extract	0.00128	0.00196
g art per 1050 ml	1.344	2.058
g of art per 950 ml	1.216	1.862
extr rate real	**54.77**	**83.87**
extr rate tot	**60.54**	**92.7**
standardized extr rate (loss 40 ml p g)	**53.62**	**82.11**

The extraction rate was lower than expected. After eight hours of extraction, only about 82% of the artemisinin present in the leaves was extracted (93% including the extract that remained on the leaves). Of the possible explanations a too high ratio of leaves to solvent turned out to be the most plausible in the light of later experiments. The fact that the leaves, which had been separated by hand from the stems and had not been milled were particularly twiggy and of unequal particle size may have added to the problem.

There were, nevertheless, a number of other observations gained from this trial:
1. The leaves absorb a lot of solvent. This is in line with reports on extraction of *artemisia* with other solvents and extractors

2. After extraction, the leaves still contain a lot of solvent. Washing and/or pressing after the extraction appear to be indispensable to achieve a higher extraction rate and, if pressing is done, the quality of the pressing would be an important consideration.

This impacts decisions as to what happens after the extraction and whether the solvent remaining on the leaves is to be recovered by other means (possibly steam stripping), or used as fuel if leaves are burnt to produce the steam for the evaporation and rectification.

3. The leaves take up a lot of physical space. A one-litre Timatic extractor can process 300 g of leaves and by extrapolation the 400 litre extractors can process 120 kg of leaves. Thus 16 extractors could extract 6 tons in 24 hours. If the extractor can be used only once every 8 hours (480 mins), the capacity of the plant would be 5.7 tons per day. A 10 hours process time would have increased the number of extractors required to process 6 tons per day beyond any reasonable limit.

4. Evaporation also posed some difficulties as the extract was rather viscous and tended to stick to the walls of the evaporator. It may be necessary to incorporate filtration prior to evaporation.

5. After drying, the extract weighed about 7-8% of the weight of the leaves. Since ethanol is less selective than some other solvents, this increase in weight was expected. Downstream purification will need to take this into account with the possibliliy of filtration prior to evaporation to reduce this bulk .

6. The spent leaves, particularly of the first batch, should have contained some artemisinin, which did not show up in the analysis of these leaves. Since it took about eight weeks after extraction before the spent leaves were tested the artemisinin may have degraded. Apparently artemisinin in the spent ethanolic leaves may decompose when the leaves are stored for a longer period. This should be kept in mind in case some conclusions on extraction efficiency are to be drawn from the analysis of spent leaves.

Second set of trials

One issue in all extraction trials is the artemisinin content of the leaves that are being subjected to the experiment. Uncertainty relating to this issue is highlighted by the fact that the leaves that were used came from one consignment of one particular farm in Tanzania which comprised 30 bags of leaves all harvested from the same field on the same day whose artemisinin content varied from 0.50% to 0.70%. Two other labs using different methods estimated the artemisinin content of the samples from the same material to be 0.48% and 0.64% respectively. So there was some variation of artemisinin content reported both from different portions of the same material analysed in the same way and from different methods of analysis.

In the second set of trials, the fresh leaves were, apparently, neither properly homogenised (mixed) nor tested before being subjected to different trials. Estimation of their content therefore had to be done from the content of the extract and the content of the spent leaves. Furthermore, a comparison with the results from the analysis performed at two sites suggests that the content of both leaves and extracts was overestimated by a constant factor of about 1:1.7. Since the extraction rates put the content of extract and leaves into relation, they could still be used to obtain a realistic picture of the outcome. Because of the problems with measurement during this set of trials, the results should, however, be read for indications of tendencies rather than as precise individual data.

Process data 3rd to 11th trial

Number of trial	DAA01-8		
Timatic Micro 1.0 Litre		Filter bag	100 micron
Compression	7 Mins	Decompression	5 Mins
ambient temperature	20° C	pressure	7 – 7.5 bar
Process time	6 hrs	(30 Cycles)	
Leaves used	Artemisia annua	Dry (8% humid)	Ground. particle size 2 mm

Calculation of results:

Number of trial	AA03	AA04	AA05
solvent ethanol	100%	80%	60%
amount of leaves used g	100	100	100
estimate amount of art available in leaves g	1.15	1.15	1.15
amount of ethanol used (ml)	1245	1223	1235
recovered liquid extract (ml)	1050	1030	1035
Artemisinin content of extract g/ml	0.00092	0.00083	0.00077
g artemisin in liquid recovered extract	0.96600	0.85490	0.79695
g artemisinin in total liquid extract	1.1454	1.01509	0.95095
extraction rate total extract TR %	**99.60**	**88.27**	**82.69**
standardised extraction rate SR %	**96.40**	**85.38**	**80.01**

The results suggest that an excellent extraction rate can be achieved with the Timatic equipment using ethanol: almost 100% of the artemisinin available in the leaves could be extracted using 100% ethanol and still 88% with 80% ethanol, - excluding the loss of extract remaining in the leaves after pressing.

Although the efficiency of the extraction is optimal, there is still a need to improve the process. The extraction time is long and the amount of leaves processed per batch is small. In addition it would be desirable to find a protocol that uses less solvent for the extraction.

There are several possible explanations why this trial yielded better results than the first trial:
- the particles of the leaves were smaller and had a more equal size,
- the amount of leaves in the extractor was smaller (1/3) and thus much more solvent was used on the leaves than in the first trial.

Contrary to some suggestions in the literature, these results demonstrated that dilution of the ethanol should be avoided and if anhydrous ethanol is not employed for cost reasons, at least dilution with water below 90% should be avoided.

Apart from ethanol, other solvents, namely hexane and isopropanol, were also tried on the same equipment under the same experimental conditions with the following results:

Number of trial	AA01	AA06	AA07
Total Process Time (hrs)	6	6	6
estimated art content of leaves g	1.15	1.23	1.15
amount of fresh leaves processed (g)	100	100	100
type of solvent	hexane 100%	isopropanol 100%	isopropanol 70%
Total Process Time (hrs)	6	6	6
volume of solvent used (ml)	1220	1218	1225
volume of extract recovered	1045	1068	995
total amount of artemisinin in extract (g/ml) after storage at room temperature	0.00073	0.00101	0.0009
g in liquid recovered extract	0.76285	1.07868	0.9
g artemisinin in total extract	0.8906	1.23018	1.1
extraction rate total extract TR %	**77.44**	**100.01**	**95.87**
standardised extraction rate SR %	**75.35**	**97.3**	**93.29**

As can be seen from the above figures, hexane is much less efficient than ethanol. Isopropanol does at least as well as ethanol and can stand more dilution than ethanol without losing its effectiveness.

The figures on "volume of extract recovered" indicate that the problem of extract clinging on the leaves is not limited to alcoholic extracts but also occurs with hexane.

Observations on the weight of dried extracts and spent leaves

After extraction for 6 hours (with the exception of one 18 hour extraction), the volume of liquid extracts harvested from different solvents, the weight of spent leaves and dried leaves and the weight of dried solid extracts were measured, giving the following results:

Weight measurements:

Solvent	leaves [g]	amount of solvent used [ml]	volume liquid extract [ml]	weight of spent leaves [g]	weight of dried spent leaves [g]	Weight spent leaves minus dried leaves (g)	weight of dried extract (g)
HEXANE100%	100	1220	1045	164.69	97.21	67,48	2.89
HEXANE100%/(18 hrs)	100	1230	1053	161.4	95.66	65.74	3.55
EtOH100%	100	1245	1050	236.46	89.34	147,12	4.7
EtOH90%	100	1205	1035	229.27	85.24	144.03	5.12
EtOH80%	100	1223	1030	236.74	76.49	160,25	7.9
EtOH60%	100	1235	1035	245.67	71.79	173.88	3.72
ISOPROPANOL100%	100	1218	1068	189.88	87.02	102,86	2.85
ISOPROPANOL70%	100	1225	995	262.65	76.04	186.61	7.83

With the exception of anhydrous isopropanol, the weight of dried alcoholic crude extracts is higher than of the dried crude hexane extracts. This may be mainly due to co-extracted sugars. Filtration prior to evaporation will remove some of the impurities of the ethanolic extracts (not the sugars), so that the weight of the dried extract will

average 4% of the weight of the dried leaves. Whether the higher weight of the ethanolic extracts has any implication for the downstream purification costs remains to be evaluated.

The spent leaves from the alcoholic extracts are heavier than those that have been treated with hexane. This difference is too large to be explained by the fact that hexane is slightly lighter than ethanol . A possible explanation is that the alcoholic extracts are more viscous and stick more to the spent leaves than those from hexane.

Stability tests

A published report that artemisinin is unstable in the presence of ethanol with possible consequences during testing of extraction processes and also for the evaporation is present in only one 20 year old article by Klayman at al[13]. In that publication they tested the stability by boiling an artemisinin extract for 48 hours at atmospheric pressure. In the process 20% of the artemisinin was destroyed, whereas no such destruction occurred when isopropanol was used. Evaluating the effects of the solvent under prolonged boiling appeared to be necessary since in their procedure extraction was done with boiling solvent and evaporation performed without the use of vacuum. Today, neither of these practices is used any more. So the question was whether the observed instability might only be seen if artemisinin is heated or boiled for a longer period or whether it also occurs when extraction or evaporation takes place at lower temperatures or when ethanolic extracts of artemisinin are stored for longer periods.

The stability of ethanolic extracts as well as other extracts was tested in the following manner: The liquid extracts were stored for 1 ½ months at room temperature and then the artemisinin content was retested.

The data suggested that there was no loss of artemisinin content of the anhydrous or aqueous ethanolic extracts when stored at room temperature for 1 1/2 months. Subsequent experiments during the large scale trials when the extracts were stored for up to four months without loss of artemisinin confirmed this conclusion.

Testing the extracts in a water bath heated to 40°C or 60°C for 24 hours did lead to some decrease of artemisinin in most of the samples. All extracts, not just ethanolic extracts may apparently loose some of their artemisinin content when subjected to elevated temperatures for a longer time. It is therefore important to keep the temperature of evaporation well below 60°C and evaporation time as short as possible.

Third set of trials

Since it appeared that the first set of experiments had unsatisfactory extraction rates because too many leaves had been processed at once, and the second set had had very high extraction rates but an unsatisfactory utilization of capacity, two more trials were conducted to find a solution between these two extremes.
The first trial was done on 200 grams of leaves with an improved version of the 1 litre Micro Timatic

[13] Daniel L. Klayman, AI J. Lin, Nancy Acton, John P. Scovill, James M. Hoch, Wilbur K. Milhous, Anthony D. Theoharides, Arthur S. Dobek: "Isolation of Artemisinin (Qinghaosu) from Artemisia annua growing in the United States", Journal of Natural products, Vol 47, No 4, pp 715-717, Jul-Aug 1984 .

Process data 12 th trial

No of Test	T200/1020/360/060207					
Equipment:	Timatic Micro 1.0 Litre - Modified - New Percolation System					
settings:	Compression	5 Mins	Decompressio	5 Mins	Percolations	5
	ambient temperature		19.5 - 21 °C	pressure	7.5 bar	
extraction:	duration	6 hrs	(36 Cycles)	filter bag	100 micron	

The following results were achieved:

No of test	T200/060207
process time hrs	6
quant leaves g	200
art content of leaves	0.65%
g of art available in 200 g leaves	1.3
solvent used	ethanol 96%
quantity of solvent used ml	1020
tot solvent recovered ml	853
g of art per 1 ml	0.00114
g of art per 853 ml	0.97242
g art per 1020 ml	1.1628
extr rate real	**74.8**
extr rate tot	**89.45**
standardised extr rate (loss 40 ml p g)	**82.43**

The efficiency of the extraction was still too low.

A further trial was therefore conducted reducing both the amount of leaves and the extraction time and introducing a wash of the spent leaves at the end of the extraction process.

The procedure was as follows: 150 grams of leaves were put in the 1 litre extractor, extraction was conducted for four hours, thereafter the leaves were pressed and the spent leaves subjected to a wash of thirty minutes. The wash was drained and again the leaves were pressed. This wash was then used for the extraction of the next batch with fresh ethanol added to ensure the extractor was full. After 4 hours extraction of the second batch, the extract was collected again and the leaves pressed.

Process data 13th and 14 th trial

No of Test	**T150/1034/240/230207/B**					
Equipment:	Timatic Micro 1.0 Litre - Modified - New Percolation System					
settings:	Compression	5 Mins	Decompression	5 Mins	Percolations	5
	ambient temperature		21 - 22.5 °C	pressure	8.5 bar	
1 extraction:	duration	4 hrs	(24 Cycles)			
wash	duration	30 mins	(3 cycles)			
2nd extraction	duration	4 hrs	(24 Cycles)			

The following results were obtained:

no of test	**T150/1034/240/230207/B**	
	serial1st batch	serial2nd batch
process time hrs	4	4
quant leaves g	150	150
art content of leaves	0.65%	0.65 %
g of art available in 150 g leaves	0.975	0.975
solvent used	ethanol 96%	ethanol 96%
quantity of solvent used ml	1034	1091
total solvent recovered ml	952	881
g of art per 1 ml	0.00078	0.00083
g of art in extract recovered ml	0.74256	0.73123
g art per total amount solvent used ml	0.806520	0.90553
TR %	**83**	**93**
SR %	**78**	**88**

As can be seen from the table above, after only 4 hours, more than three quarters of the artemisinin had been extracted and an additional wash of only thirty minutes raised the standardised extraction rate by another 10%, which showed up as additional artemisinin extracted from the second batch.

Since the second batch could have been subjected to a wash as well, adding another 8- 10% to the proportion of artemisinin extracted. The extraction from both batches would have given an extraction rate of close to 90%.

The extraction time of 4.5 hours including the wash was too short to achieve the maximum extraction rate. Either serial extraction with more steps or a longer extraction time would be necessary.

The final trial was performed extracting 100 g of leaves for 7 hours with 1098 ml of ethanol 96% and using the extract collected from draining and pressing of the leaves on a second batch of 100 g of leaves together with an addition of 144 ml of solvent. The results suggested that the total extraction rate (without losses of extracts on spent leaves) was close to 100% and that a standardised extraction rate of about 93% could be achieved. For this particular trial, however, full leaf analysis was not possible, so this result needs to be treated with caution. Assuming this result is reproducible, solvent use can be reduced to 5 litres of ethanol per kilogram of leaves.
While further work remains, it can be concluded that it is possible to use the Timatic extractors for an efficient extraction of artemisinin. Solvent use can has be minimised and the extractors are well suited for small scale extraction. An additional benefit is that these extractors are versatile allowing extraction of other plants and use of other solvents.

For large scale extraction, the extractors are not suitable. The largest Timatic extractor only holds 400 litres of solvent. It would take a large number of extractors to process several tons per day – a prohibitive number in terms of capital costs and labour required. An additional problem is that the normal way of filling and emptying a Timatic extractor is by using a filter bag to hold the leaves. The extractor is filled and emptied by handling this bag. If there are larger extractors and more extractors involved, this method would not be suitable because of the danger of escaping ethanol vapour during the emptying of the extractor. It would therefore be necessary to redesign the extractor.

There are considerable economies of scale regarding the rest of the equipment needed for the ethanolic extraction such as the evaporator and the rectification column and there are economies of scale in relationship to the skilled personnel and the management required for an extraction unit, so that the ideal size appears to be that of a unit that can process 6 tons of leaves in 24 hours. For this reason, larger extractors of a different design where one or two vessels would be enough to handle all the leaves appeared to be preferable.

Conclusions from the small scale trials

The following could be learnt from the small scale trials:

- Ethanol is a more efficient solvent than hexane.
- The optimal extraction time is around 6-7 hours, a ratio of leaves to solvent (w/v) of around 1:5 is possible but then the extract needs to be washed.
- The improved Timatic extractor is suitable for the extraction of *artemisia annua* in a small scale extraction facility.
- Ethanol works better if dilution with water does not exceed 10%.
- The leaves absorb solvent which remains with the spent leaves and has to be removed by efficient pressing. Pressing is therefore an important variable in the extraction process.
- There is no problem of instability of artemisinin contained in ethanolic extracts, provided these extracts are not diluted or heated over extended periods.
- Ethanol is less selective: The extracts that come from ethanol are heavier than those from hexane. A large proportion of these additional impurities can be presumed to be various sugars.
-

Larger scale trials

Searching for the right kind of extractor

Inquiries into the use of counter-current continuous extractors with two different suppliers of such equipment showed that the most common models of counter-current extractors with a screw conveyor were not suitable for East African leaves which contained many very small particles. Counter-current continuous extractors that use other types of conveyors may either not satisfy the requirements of explosion proof operations or require a much expensive pipe work. It was therefore decided that a batch type extractor would be more suitable.

The solvent can come in contact with leaves in various ways: by maceration (soaking), by percolation (forcing the solvent through the leaves), or by a process where the leaves are only washed on the outside by stirring . Since artemisinin is only present in the epidermis of the leaves, the latter appeared to be the most appropriate method.

It appeared that the best solution would be a large paddle stirrer type of extractor which can take several tons of leaves at once and can be allowed to run as long as necessary. Inside the extractor the solvent and the leaves are turned over by customised stirrer paddles so that maximum contact between the surface of the leaves and the solvent is assured. Such large simple agitator extractors are used extensively in the fermentation and the extraction industry[14]. As there was no company that

[14] for instance, William Ransom & Sons PLC, Hitchen, Herts, England, use them. See also page 625 of McCabe, Smith & Harriott: Unit Operations of Chemical Engineering; McGraw-Hill International; in the section on " Leaching, Extraction Equipment (Mixer-Settlers) " which states :"A tank containing a turbine or propeller agitator is most common"

offered such an extractor for trials it was necessary to build a trial extractor specifically for the artemisia project. A 50-litre extractor and a 20-litre extractor were built and gradually optimised for the task which they were to perform.

Conditions of the trials

Leaves from Tanzania were purchased from East African Botanicals Limited. The leaves had been harvested by the so-called tractor method, that is, by separating stems and leaves by driving a tractor over them followed by hand sieving. As a result, flakes of about 1-3 mm length are produced which require no further milling or sieving.

The leaves contained a considerable amount of sand, which later proved cumbersome during the draining of the extracts. Reducing the sand by making sure that threshing grounds are clean and/or by garbling the leaves properly prior to bagging is recommended.

It is possible that extraction efficiency might be enhanced by milling the leaves to a uniform size of about 1 mm, although this would probably also increase the amount of impurities that can be found in the extract. It was decided not to include the issue of optimising extraction by milling in the trials which are reported here.

The leaves were thoroughly homogenised (mixed) by hand and a sample of each homogenised batch of leaves was sent for testing.

The leaves turned out to have 0.65-0.68% artemisinin content., a disappointingly low rate, since experienced farmers in the region are able to achieve artemisinin contents above 93% on a regular basis[15].

The composition of the solvent used was 97.6% ethanol, and a minimum of 2% ethyl acetate and 0.1% isopropanol. The latter two are quoted as minimum figures. All the large scale trials were all performed with anhydrous solvent. For cost reasons, however, 96% alcohol will be employed in a commercial facility. Additional trials confirmed that there is no noticeable difference in the extraction efficiency of this anhydrous solvent and ethanol diluted with water down to 92%, so that the same results can be achieved with a slightly more diluted and less expensive solvent.

For each trial the extractor was filled with 50, 40, 30 or 20 litres of solvent and the amount of leaves required for the particular trial (for instance, 5 kg of leaves and 50 litres of solvent, for a 1:10 ratio of leaves to ethanol). After the completion of the desired extraction time, a sample of the extract was taken for testing of the artemisinin content and the rest of the extract was harvested and measured. During the first trials the spent leaves were weighed before and after pressing and after drying. In later trials the amount of solvent to be collected by pressing was merely estimated.

Trials on extraction efficiency

The first purpose of the extraction trials was to determine:

- the optimum ratio between solvent and leaves,
- the optimum extraction time,
- the optimum extraction temperature,
- the optimum design of the stirrer paddles,
- and the maximum extraction rate that is possible.

[15] Information from ABE Ltd and from individual farmers.

12 runs with different quantities of leaves and solvent, at different temperatures and with differently designed stirrers were conducted, and extraction times for these runs were monitored by taking samples at different intervals. Extraction efficiency was calculated assuming that all the solvent employed could have been recovered in the extract (total extraction rate) and assuming that 0.40 litres of extract per kg of fresh leaves remained entrained on the spent leaves. (standardised extraction rate).

Below are the results:

run	ratio 1 kg leaves/ liters EtOH	temp	stirrer paddles	Duration of extraction in hours	TR %	amount of solvent remaining on leaf after draining per kg of fresh leaves	loss of extract if after pressing 0.40 litres of extract per 1 kg of fresh leaf remain un-recovered %	SR if after pressing 0.40 litres of extract per kg of fresh leaves remain un-recovered %
1	10	10° C	1	8	86	2.20	4.00	82
2	10	25° C	1	8	89	2.00	4.00	85
3	10	25° C	2	4	92	3.33	4.00	88
3	10	25°C	2	6	98	3.33	4.00	94
3	10	25°C	2	8	100	3.33	4.00	96
4	6.67	25°C	2	6	94	2.89	6.00	88
4	6.67	25°C	2	8	96	2.89	6.00	90
6	7.5	25°C	3	7	100	2.25	5.33	98
11	7.46	25°C	4	6	98	1.94	5.36	92
11	7.46	25°C	4	7	100	1.94	5.36	94
12	7.5	15°C	5	6	98	2.25	5.33	92
12	7.5	15° C	5	7	100	2.25	5.33	94

Results of extraction trials, consolidated table.
(* paddles 1 – 5 = 5 different designs of stirrers)

As can be concluded from the figures above, improvement of the design of the stirrer paddles (five different designs were tested) continuously improved extraction efficiency allowing a reduction in extraction time. During the last tests the optimum time was 6-7 hours. As far as the optimal relationship between leaves and solvent is concerned, a relation of about 1 to 7.5 (for example, 4 kg of leaves to 30 litres of solvent) proved to be the best attainable.The improvement in design also did away with the need for heating the solvent. Temperatures between 15°C and 25°C allowed efficient extraction.

Using the technique which Rodrigues at al (see Annex 2) have suggested to reduce extraction time, namely to split the extraction into 3 stages of 1.5 hours each plus a concluding wash, collecting the extract and starting with fresh solvent at the beginning of each stage, is inconvenient for larger scale extraction. Furthermore, twice as much solvent would have been needed (17.52 litres for 1 kg of leaves), and the draining of the extract after each stage would have required more than 30 minutes, so that total extraction time would have been increased by more than 6 hours.

Trials with washes

Since the extraction trials showed that it was possible to increase the artemisinin concentration of the extract by using more leaves per litre of solvent, the fact that so

much of the extract was retained on the leaves after draining became more of an issue. The problem would not be how to recover the solvent for further use: this can be done by heating the leaves in a closed environment at the end of the extraction. The heat needed for this operation would, however, destroy the artemisinin which is still present in the extract. Data were needed on how to remove as much extract as possible from the leaves before the final step of removing the solvent by heating. The question was, whether one wash was needed or two washes and whether washes would make pressing superfluous or not.

The extraction protocol suggested by Rodrigues et al (See Annex 2) recommends one final wash. The authors of EXTEN study (see appendix) apparently grappled with the same problem, tried two washes and experimented with even a third wash using reduced amounts of solvent.

11 trials were made to investigate washes of the extracted leaves.

reuse of wash or extracts; art content of leaves : 0,66%								
Run no	quantity of extract or wash litres	art in extract or wash gram	kg of leaves involved	total art in gram available	duration hrs	content of extract harvested g/l	art in total extract in g	TR
8	40	30.40	6.00	70.00	6.00	1.65	66.00	94
9	30	7.80	4.50	37.50	6.00	1.26	37.80	101
10	30	49.50	4.50	79.20	6.00	2.60	78.00	98
13/1'	30	27.00	4.00	53.40	6.00	1.72	51.60	97
13/2'	30	27.00	4.00	53.40	7.00	1.74	52.20	98

The following could be learned about washes:

- Washes become necessary when less solvent is used for the first extraction so that the artemisinin in the extract becomes more concentrated.
- The efficiency of the wash is directly related to the degree of dilution of the entrained extract. The greater the quantity of solvent used in the wash in relationship to the extract entrained on the spent leaves, the higher the extraction rate of the wash. Care has to be taken, however, that one does not spend on the wash as much solvent as was saved on the extraction.
- The optimal time for the washes (using optimised stirring paddles: type 5) was one hour.
- The liquid used in the wash does not add to the quantity of entrained liquid on the leaves. If, after extraction and draining of the extract, a certain quantity of solvent is added for the wash, the same quantity can be removed by draining afterwards without problems.
- Using a second wash is uneconomic. Too much solvent is needed for the second wash in relationship to the rather small amounts of artemisinin that can be recovered.
- When the leaves were only drained and not pressed after the last wash, about 10% of the artemisinin available in the wash was lost. Washing alone cannot increase the overall extraction rate above 90%, because too much extract remains on the spent leaves after draining. Pressing is still necessary.

Trials on serial extraction

Rodrigues et al developed a scheme for the erhanolic extraction of artemisia leaves, which they called "serial extraction", which allowed for considerable savings in the amount of solvent employed, by using the extract of the first batch without evaporation

as a solvent on the second batch. They extracted each batch three times for 1.5 hours only, followed by a wash..

This example led to the question whether extracts and washes could be used on further batches of leaves even if extraction takes place in only one step of 6 to 7 hours and not in three steps of 1.5 hours each.
As can be seen from the following table, these trials were quite successful:

reuse of wash or extracts; art content of leaves : 0,66%								
Run no	quantity of extract or wash litres	art in extract or wash gram	kg of leaves involved	total art in gram available	duration hrs	content of extract harvested g/l	art in total extract in g	TR
8	40	30.40	6.00	70.00	6.00	1.65	66.00	94
9	30	7.80	4.50	37.50	6.00	1.26	37.80	101
10	30	49.50	4.50	79.20	6.00	2.60	78.00	98
13/1'	30	27.00	4.00	53.40	6.00	1.72	51.60	97
13/2'	30	27.00	4.00	53.40	7.00	1.74	52.20	98

It is apparent that most of the artemisinin that came from batch one turned up in batch two, when the extract that had now gone through both batches was collected.

Since the optimum ratio of leaves to solvent for one batch had been 1 kg to 7.5 litres, by using serial extraction it would be possible to reduce the ratio of leaves to solvent to 1 kg to 3.75 litres of solvent. The problem that needs to be addressed then is how to get the extract, particularly the concentrated extract of the second batch in each cycle, off the spent leaves. If washing is employed then the total amount of solvent used would rise again because additional solvent is needed for the wash.

Finding the optimal protocol

Using data from the trials described above, the following three protocols were developed and subjected to experimentation: The examples are given for 4 kg batches, but this figure could be scaled up to the desired quantity to be processed.

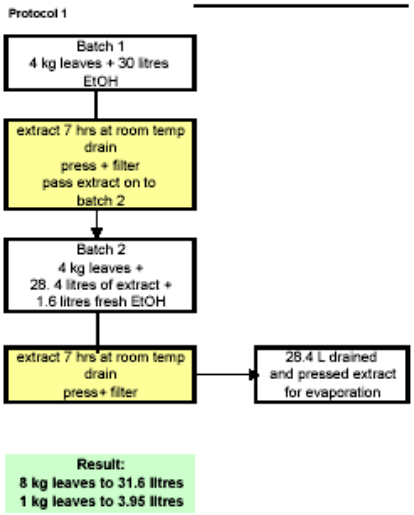
The first protocol used the extract from the first batch as solvent for the second batch. The total extraction rate was high, but too much artemisinin was lost in the concentrated extract which remained on the leaves of the second batch after pressing.

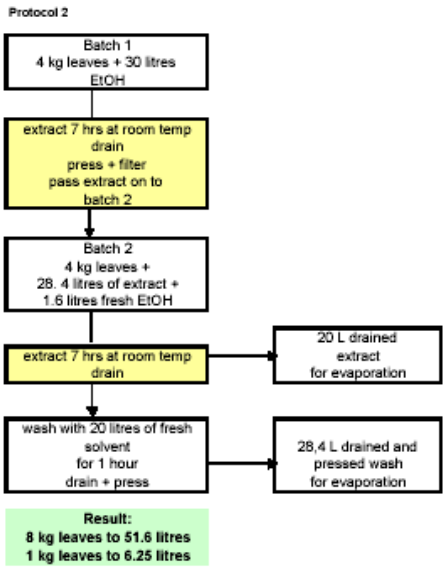
Therefore, in the second protocol, a wash of the second batch was introduced. However in this case too much solvent was employed.

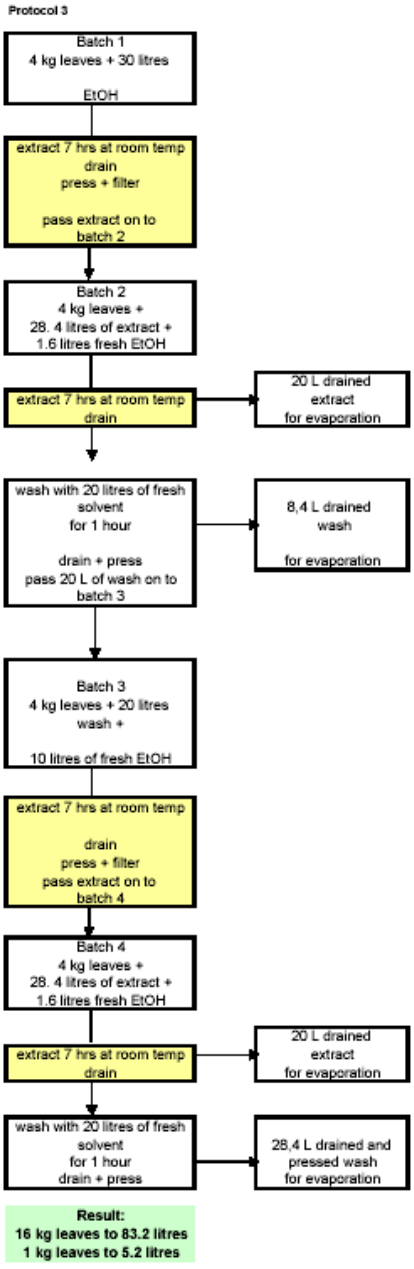
In protocol three, the wash of the second batch was used as a solvent for the third batch and the extract of the third batch is then used as solvent for the fourth batch. Protocol 3 is, however, rather complicated.

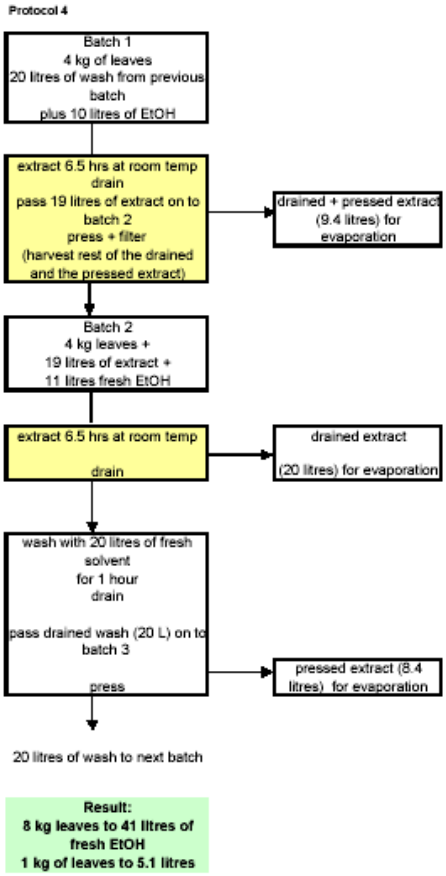
Instead, it is possible to introduce a circular process as shown in protocol 4. In this protocol, the second batch is extracted with the drained wash of the previous batch, to which solvent is added to fill the extractor. About 95% of the drained extract of batch one is passed on to batch two and some solvent added to fill the extractor a second time. After draining batch two, the extractor is re-filled with fresh solvent and the leaves are washed. The wash is drained and used for the extraction of the next first batch and so on.

See the diagrams below for the protocols:.


Protocol 1
Batch 1
4 kg leaves + 30 litres EtOH
extract 7 hrs at room temp
drain
press + filter
pass extract on to batch 2
Batch 2
4 kg leaves +
28. 4 litres of extract +
1.6 litres fresh EtOH
extract 7 hrs at room temp
drain
press+ filter
28.4 L drained and pressed extract for evaporation
Result:
8 kg leaves to 31.6 litres
1 kg leaves to 3.95 litres


Protocol 2
Batch 1
4 kg leaves + 30 litres EtOH
extract 7 hrs at room temp
drain
press + filter
pass extract on to batch 2
Batch 2
4 kg leaves +
28. 4 litres of extract +
1.6 litres fresh EtOH
extract 7 hrs at room temp
drain
20 L drained extract for evaporation
wash with 20 litres of fresh solvent
for 1 hour
drain + press
28,4 L drained and pressed wash for evaporation
Result:
8 kg leaves to 51.6 litres
1 kg leaves to 6.25 litres


Protocol 3
Batch 1
4 kg leaves + 30 litres
EtOH
extract 7 hrs at room temp
drain
press + filter
pass extract on to batch 2
Batch 2
4 kg leaves +
28. 4 litres of extract +
1.6 litres fresh EtOH
extract 7 hrs at room temp
drain
20 L drained extract for evaporation
wash with 20 litres of fresh solvent
for 1 hour
drain + press
pass 20 L of wash on to batch 3
8,4 L drained wash for evaporation
Batch 3
4 kg leaves + 20 litres wash +
10 litres of fresh EtOH
extract 7 hrs at room temp
drain
press + filter
pass extract on to batch 4
Batch 4
4 kg leaves +
28. 4 litres of extract +
1.6 litres fresh EtOH
extract 7 hrs at room temp
drain
20 L drained extract for evaporation
wash with 20 litres of fresh solvent
for 1 hour
drain + press
28,4 L drained and pressed wash for evaporation
Result:
16 kg leaves to 83.2 litres
1 kg leaves to 5.2 litres


Protocol 4
Batch 1
4 kg of leaves
20 litres of wash from previous batch
plus 10 litres of EtOH
extract 6.5 hrs at room temp
drain
pass 19 litres of extract on to batch 2
press + filter
(harvest rest of the drained and the pressed extract)
drained + pressed extract (9.4 litres) for evaporation
Batch 2
4 kg leaves +
19 litres of extract +
11 litres fresh EtOH
extract 6.5 hrs at room temp
drain
drained extract
(20 litres) for evaporation
wash with 20 litres of fresh solvent
for 1 hour
drain
pass drained wash (20 L) on to batch 3
press
pressed extract (8.4 litres) for evaporation
20 litres of wash to next batch
Result:
8 kg leaves to 41 litres of fresh EtOH
1 kg of leaves to 5.1 litres

Trials with these different protocols suggest that if pressing can reduce the amount of extract that remains entrained to about 0.45 litres per kg of leaves, an extraction rate of about 91 % can be expected from protocol 1, and up to 94 % from protocols 2, 3 and 4. Because of the lower use of solvent and its greater simplicity, protocol 4 appears to be the best.

A ratio of about 1: 5.2 between fresh leaves and solvent (w/v) compares favourable with the 1:10 or even more reported by some extraction companies working with hexane. Another advantage is an extraction cycle of about 6.5 hours per batch, compared to 16 hours which is common in extraction with hexane.

Trials on pressing

The type of press needed is a screw press.

Ponndorf screw presses are the best know presses of this kind in Europe, since this company invented the screw press many years ago. Ponndorf Maschinenfabrik GmbH answered to our inquiry that they had no data on the efficiency of their press on different herbs, but on nettles the press had reduced the moisture of the spent leaves to 50%. It was impossible to do trials at the company site and the price of their press is relatively high.
KC Co. Ltd, a company producing screw presses in WA, USA, did laboratory tests on the artemisia leaves, which suggested that it might be possible to reduce the amount of liquid entrained to 0.35 litres per kg of fresh leaves. Their method of testing did, however, not produce very reliable results.
Finally, an extraction firm in France (NATEVA), who extract herbs with ethanol and have a customised hydraulic screw press, performed two larger scale trials. In the first trial the leaves were macerated for 16 days, rather than extracted. In the second trial they were only macerated for 14 hours thus coming closer to reproducing the extraction procedure used with the SPT extractor. The following were the results of the pressing:

Duration of Maceration	16 days	14 hours
Quantity of plants	20 kg	20 kg
Water contained	1.9 kg	1.9 kg
Quantity of 96,5° alcohol	81 litres	81 litres
Pressing time	30 mn	30 mn
Quantity of spent leaves	24,3 kg	26,3 kg
Ethanol entrained in spent leaves	4.3 kg	6.3 kg
- in litres	5.35 litres	7.83 litres
Ethanol entrained in pressed leaves per kg of fresh leaves	**0.31 litres**	**0.41 litres**
Quantity of extract harvested	71 litres	69 litres
Extract alcoholic grade	90°	92.5°
Loss of alcohol	14.27 litres	14.34 litres
Due to evaporation	9.11 litres	6.78 litres
Extract density		0,81

In the first test, 10 litres, or about 0.50 litres of solvent per kg of fresh leaves remained unrecoverable. Of this, however, only 0.31 litre per kg of fresh leaves was entrained in the spent leaves. The remainder of the missing solvent escaped by evaporation during

the long maceration of the leaves prior to pressing. With extraction, where the vapours cannot escape, this does not occur. In the second trial - with a very short maceration time – more solvent remained entrained in the leaves (0.41 litres per kg of fresh leaves). Again, some of the solvent that could not be recovered, evaporated during maceration. The original expectation, that only about 0.40 litres of extract per kg of fresh leaves will remain in the leaves and the rest will be recovered, appears therefore to be realistic.

A loss of 0.45 litres of extract per kg of fresh leaves was used in calculation of the mass data.
(It should also be noted that maceration transports a lot more plant liquid into the solvent than extraction by stirring, so a significantly higher alcohol content of the extract can be expected from the SPT extractor.)

The ethanol that does remains on the leaves after pressing will not be lost but will be recovered by heating of the spent leaves. Only the artemisinin that is still in the extract on the pressed leaves will be lost during the heating process.

Evaporation and Filtration

Evaporation has to be done in several stages.

Evaporation of the extract that contains a considerable amount of impurities is difficult and also makes subsequent purification more laborious. Filtering is therefore required.

Following the first stage evaporation, during which the extract is concentrated ten fold, a considerable amount of impurities, such as oils and sugars as well as some fine soil and fine leaf solids precipitate out.

Trials on a laboratory centrifuge established that these solids could be removed from the extract.

In the actual full scale process a decanter type centrifuge will be used for this stage. These centrifuges are rotating devices. They consist of an outer drum and an inner drum. Both are cone shaped. The feed with the solids enters into the space between the two drums. The outer drum rotates at a speed of up to 5,000rpm and the solids in the feed are pressed onto the inner wall of the outer drum. The liquid containing lighter impurities creates a layer above the heavier solids. An Archimedean type screw on the inner drum pushes the layer of heavier solids to an outlet at the narrow end where they fall out. The liquid flows to the back end of the drums where it too falls out.

This is a continuous device and does not suffer the problems of blocking that other filter mechanisms, with cloths etc would suffer on this application. The centrifuge can be cleaned easily and throughput is easily regulated and adjusted.

The clear extract can then be further concentrated by evaporation.

Costs of rectification

After the trials established the amount of solvent required for the extraction and the amount lost on the leaves after pressing, the amount that needs to be evaporated and rectified could also be estimated.
If protocol 3 is used, 4775 litres of mixed extracts and washes have to be evaporated for every ton of artemisia processed. (The remainder of the utilised solvent is recovered from heating of the leaves). This evaporation would cost about 25.80 Euros per day. Included in this figure are also the costs for drying the extract and heating the leaves.

The liquid that is evaporated consists of about 52% concentrated extract and 48% wash. Analysis of the extracts from protocol 3 revealed that the SPT extractor, in which the solvent circulates around the surface of the leaves rather then penetrating them, does not co-extract much plant liquor, since the latter mainly resides in the veins of the leaves. Dilution of the ethanol ranged from 1% to 2.2 % during a single extraction. Since each extract was used twice, the dilution increased to about 2 to 4 % after the second extraction. The washes did not extract any significant amount of plant liquid, so the mixture between the extract and the wash that is evaporated will contain only about 1 to 2% of plant liquid. Because of this, rectification can be done simply by linking the rectification column to the evaporator and stripping off the excess plant fluid continuously. The rectification column will derive its energy from the evaporator stage. Because of this, costs will be much less than with a stand-alone rectification column. The cost would be approximately 6.20 Euros per ton of leaves processed and would thus increase the total costs, compared to evaporation alone, by about 25%.

Costs of evaporation and rectification might be reduced even further by using a boiler for producing the steam in which the spent artemisia leaves can be burnt, preferably mixed with some saw dust if a saw mill is in the vicinity of the extraction facility. The addition of saw dust would be helpful since the calorific value of artemisia is not very high.

In terms of capital costs, the evaporator will add about 10 to 11% to total capital costs and the rectification column will add further 6-8%.

As can be seen from these figures, the costs of rectification are not as prohibitive as some experts, whom we interviewed, assumed. Indeed, since ethanolic extraction can be done with less solvent per ton of leaves, the combined costs of extraction and rectification may, in fact, be lower than the evaporation costs of facilities that use hexane as a solvent.

Summary: Conclusions regarding the potential of ethanolic extraction

The study presented here comes to the following conclusions:

1. Ethanol is less dangerous to the workers and the environment than hexane. The danger that the solvent may be abused as a spirit can be contained by spiking it with a small amount of ethyl acetate or isopropanol.
2. Artemisinin is stable in ethanol.
3. 95% Ethanol extracts artemisinin more efficiently than ethanol that contains more than 10% water.
4. Two types of extractors were successfully tested, the Timatic extractor for small scale extraction and the customised SPT extractor for larger scale extraction. With these extractors it is possible to extract 95% of the artemisinin available in the leaves in 6-7 hours. These efficient extractors can work at room temperature.
5. It is possible to economise on solvent by using the extract from one batch as solvent on the next batch. In this case the spent leaves have to be washed, but the wash can also be reused for extraction. A protocol was worked out which reduces the amount of solvent required to less than 6 litres per kg of leaves.
6. By pressing with a suitable hydraulic press it is possible to reduce the extract that remains on the spent leaves to 0.45 litres per kg of leaves. The solvent entrained on the spent leaves after pressing can be recovered by heating (toasting) the leaves.
7. The SPT extractor does not co-extract much plant liquid. This reduces the costs of rectification.
8. Despite the need for rectification, ethanolic extraction is more economic than extraction with hexane. Extraction with ethanol is economically feasible. If an

extraction facility with the capacity to process 6 tons of leaves per day using the technology described in this study were erected in Tanzania, it would be possible to reduce the price of artemisinin to about 320 USD per kg and still achieve a return of 15% on equity before tax. (See Annex 6).

9. Ethanol does co-extract more, mainly polar, impurities than hexane. Judging from the available literature, a relatively inexpensive purification process that involves chromatography without the use of silica gel can be used for purification. It would lead to a loss of 20% of the extracted artemisinin. While this would still make ethanolic extraction more economic than extraction with hexane, the search for other, more efficient, extraction protocols should be undertaken.

Annex No 1 : Review of literature on ethanolic extraction

The most detailed description of an extraction protocol of ethanolic extraction has been presented by Rodrigues et al[16] based on laboratory scale experiments. They developed a scheme for the extraction of artemisia leaves they called "serial extraction". Ground artemisia leaves with a particle size of less than 1 mm were extracted with fresh solvent in three stages of 1.5 hours each (total extraction time 4.5 hours) with 96% ethanol, draining and filtering the extract after each stage. This was followed by a short wash. Afterwards the filtered extract of the first stage of the first batch was used, with the addition of 30% fresh ethanol, as a solvent on the first stage of the second batch, the extract of the second stage of the first batch was used, with the addition of 30% fresh ethanol, on the second stage of the second batch like a solvent and the extract from the third stage of the first batch was also used, with the addition of 30% fresh ethanol, as a solvent for the third stage of the second batch. Finally the wash of batch one was used, with some addition of fresh solvent, for the wash of batch two. In this manner the amount of solvent needed could be halved to 6.3 litres per kilogram of leaves plus 1 litre for washing.

Their purification protocol is described in the section on purification. The extraction rate prior to purification was 91%.

Kumar et al [17] did a number of lab scale experiments comparing extraction with anhydrous ethanol, methanol and hexane, as well as extraction with aqueous (20%) ethanol, methanol and hexane. In every case the anhydrous solvents were more effective than the aqueous solvents. Furthermore, some decomposition of artemisinin was observed in the aqueous solvents. Of the three, ethanol was the most efficient, followed by methanol, with hexane giving the lowest yield. They also experimented with extraction temperatures for ethanolic extraction, heating the solvent to 20, 30, 40 and 50° C. with 30° C giving the best result.

The relationship of leaves to solvent was also investigated. They found that a ratio of 1:10 (w/v) gave a slightly higher yield than 1:6 (w/v). They extracted in four stages of four hours each, using the ratios of leaves to solvent described above. The total amount of solvent required and the total extraction time were not reported. The artemisinin content of the leaves prior to extraction and the extraction rate prior to purification are also not reported. Their purification process will be described in the section dealing with purification.

Jiashou Zhang et al[18] propose to leach the leaves in aqueous ethanol (30%) and then percolate the solvent into gasoline to which 30% benzene or ethyl acetate is added. No extraction rate given. Benzene is very poisonous and carcinogenic and therefore not an acceptable solvent.

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[16] Ferreira Rodrigues, Alexandre Rodney ; Foglio, Mary Ann ; Boaventura Júnior, Sinesio; da Silva, Adriana; Garcia Rehder, Santos; Garcia Rehder, Vera; (CPPQ-UniCamp,Brazil);: Optimazao do Processo de extracao et isolamento do antimalarico Artemisinina a partir de Artemisia Annua L. ; Quim Nova, Vol 29, 2, 368 –372, 200;

[17] Kumar, Sushil; Gupta, Shiv Kumar; Gupta, Madan Mohan; Jain, Dharam Chand; Khanuja, Suman Preet Singh; Kahol, Atul Prakash; Singh, Digvijay; Ram, Govind; Process for isolatine artemisinin from Artemisia annua; US Patent 6685972, Feb 2004

[18] Jiashuo Zhang (CN); Dongwu Fan (CN); Xioben Ma (CN) ,Method for extraction of artemisinin; Chinese Patent 1092073

In a study financed by the EU[19], the possibility of extracting various botanicals with ethanol and then purifying with supercritical carbon dioxide in one continuous process was examined. The botanicals tried included *artemisia annua*. Experimenting with milling methods, they came to the conclusion that it was important to use mills that produce homogenous particles. They proposed a two stage serial extraction followed by a wash: The extract of the second stage of the first batch was used as solvent for the first stage of the second batch. The liquid from the wash of the first batch was added to it. Extraction times are not reported. The report claims that both the extraction protocol and the purification with ScCO2 were successful but no extraction rates are reported.

Annex No 2 : Safety data on n-hexane and ethanol

Hexane is more explosive and also more dangerous to human health and the environment than ethanol.

The differences can be explained in the following manner :

1. Flammability

As far the danger of explosions is concerned, both ethanol and n-hexane are classed as very flammable. Equipment, engineering and work processes have to be designed accordingly. They both can form vapours and produce explosive mixtures with air. N-hexane is, however, more explosive than ethanol [20]:

	Flash point (method tcc)	Flammable limits in air, lower point	Flammable limits in air, higher point	Vapour density vs air	Evapo-ration rate [15]
ethanol	13° C	3.3 %	19 %	1.6	1.4
n-hexane	- 22° C	1.1 %	7.7%	3.0 – 3.8	8.3-9

The above indicators imply that if there are leaks or spills, hexane will evaporate earlier and faster and the vapour can creep rather rapidly along the floor and may ignite. Apparently, industrial accidents involving the explosion of hexane still occur even in industrialised countries despite increasing efforts to prevent them [21]. There a no reports on the internet of such accidents involving ethanol.

2. Occupational Health

Problems related to occupational health mainly arise from inhalation of vapours of the solvent and from accidental skin contact. Ingestion would be an unlikely event. While

[19] EXTEN; Volume extraction and encapsulation of substances used as flavour chemicals, pharmaceutical raw substances, biochemicals and enzymatic systems; BioMatNet database: European Union, -*FAIR-CT96-2003*

[20] Source: OSHA at http://www.osha.gov/SLTC/helthguidelines (accessed May 2007)

[21] For example, a google search with the keywords "explosion + hexane" yielded for the years 2002 – 2007 for the USA alone 4 reports of industrial accidents involving hexane explosions.
http://www.acusafe.com/Newsletter/Stories/0202News-MonthlyIncidents.htm,
http://nutiva.com/about/media/2003_08_29.php
http://www.tntmirror.com/friday/2006/jan06/story01.htm
http://www.blueridgenow.com/article/20070807/NEWS/70807004/0
(downloaded on Dec 3, 2007)

both ethanol and hexane can pose a danger if inhaled, the threshold values differ significantly: The maximum 8 hour time weighed average exposure at the workplace to hexane vapours in the air allowed in Australia is 50 ppm [22], whereas for ethanol it would be 1000 ppm. The US standards (OSHA) still allow for 500 ppm of n-hexane for average exposure, but there are reports that already at that level, neuropathic symptoms such as numbness and muscle weakness in the lower extremities can be observed as an occupational disease. The US National Institute for Occupational Safety (NIOSH) recommends a limit of 50 ppm, which is also the standard in Australia.

The European Commission has recently issued a directive [23] which sets the limit value of exposure to hexane at 20 ppm.

Dermal contact with n-hexane can produce immediate irritation, whereas skin contact with small quantities of ethanol merely leads to a dry skin. It is for this reason that the European rules on "Dangerous Substances Classification and Labelling" require n-hexane to be labelled as an irritant (Xn) [24], while this is not the case for ethanol.

3. Impact on the environment

Since hexane is more poisonous, the danger of enviromental contamination from traces of solvent remaining on the spent leaves, if they are not burnt on site, is somewhat greater. However, since both hexane and ethanol evaporate and degrade easily, they do not usually become significant sources of soil or water pollution. If ethanol does seep into the groundwater it is much less dangerous not only because it is less poisonous but also because, unlike hexane, it is completely miscible with water and is thus rapidly diluted and biodegraded [25]. In the air, hexane reacts with photo-chemically produced hydroxyl radicals and produces ground level ozone [26]. Hexane is included in the list of 189 toxic chemicals which are on the TRI (toxic release inventory) of the U.S. Environmental Protection Agency (EPA) as a HAP (hazardous air pollutant)[27]. It must be added, that ethanol, which is not listed as a HAP, also pollutes the air by decomposing into CO2 thus contributing to global warming.

4. Overall rating:

[22] ppm: parts per million by volume of air (ml/m³)

[23] Commission Directive 2006/15/EC, of 7 February 2006,Official Journal L 038 , 09/02/2006 P. 0036 - 0039

[24] see Material Safety Data Sheet NORMAL HEXANE, PURE issued by Chevron Phillips, Chemical company at http.//www.cpchem.com/enu/NORMAL_HEXANE_PURE.asp, (accessed May 2007)

[25] Rice, David R.; Environmental Protection Department, Lawrence Livermore National Laboratory; Briefing to the California Environmental Policy Council Sacramento, California; Potential ground and surface water impacts associated with the use of ethanol as a fuel oxygenate; January 18, 2000, http:// www-envirinfo.llnl.gov/ECBG_final.pdf (accessed May 2007), NCGA (National Corn Growers Association; Ethanol is safe in soil and groundwater; http.// www.ncga.com/ethanol/environment/soilGroundwater.asp (accessed May 2007

[26] National Center for Manufacturing Sciences (NCMS), Solv-DB http://solvdb.ncms.org/ncmsenvr.idc?solvno=00110543A (accesses May 2007)

[27] Refer to Inform, Vol. 9, No.7, July 1998:p 708.

NPFA (U.S. National Fire Protection Association) has done the following rating

	Health (scale 0 – 4) 1 = slightly hazardous	Flammability (scale 0 – 4) 3 = Flash point below 100 F	Reactivity (scale 0 – 4) 1 = unstable when heated
ethanol	0	3	0
n-hexane	1	3	1

The Australian National Pollutant Inventory (NPI) [1] has rated health and environmental hazards caused by ethanol and n-hexane in the following manner:

	Human health hazard (scale :0-3) 0 = negligible 1 = harmful 2 = medium hazard 3 = very high hazard	Environmental Hazard (scale: 0 – 3) 0 = negligible 1 = harmful 2 = medium hazard 3 = very high hazard	Total Hazard Score (scale: 0 – 6)	Ranking in 400 industrial substances examined (scale 1-208)
ethanol	1.2	1.3	2.5	86
n-hexane	1.3	1.7	3.0	89

Annex No 3: List of equipment required for an extraction unit

List of Equipment

1	Drying equipment for leaves
2	Equipment for milling and sieving (Comil)
3	Extractors (2+1 standby)
4	Air Compression system and pipe-work
5	Simple filter press
6	Hydraulic screw press
7	Wiped thin film evaporator
8	Mechanical mixer
9	Ethanol rectification column
10	Centrifuge for crude extract
11	Small evaporator for crude extract
12	Dryer for crude extract
13	Desolventiser for spent leaves
14	Optional: Boiler that can use spent leaves to produce steam
15	Other plant services: chilled water etc.
16	Stainless steel hoppers & storage tanks
17	Pumps (5 stainless steel centrifugal flameproof motors)
18	Screw conveyors
19	Purification equipment and recrystallisation unit
20	Various smaller items
21	Standby generator

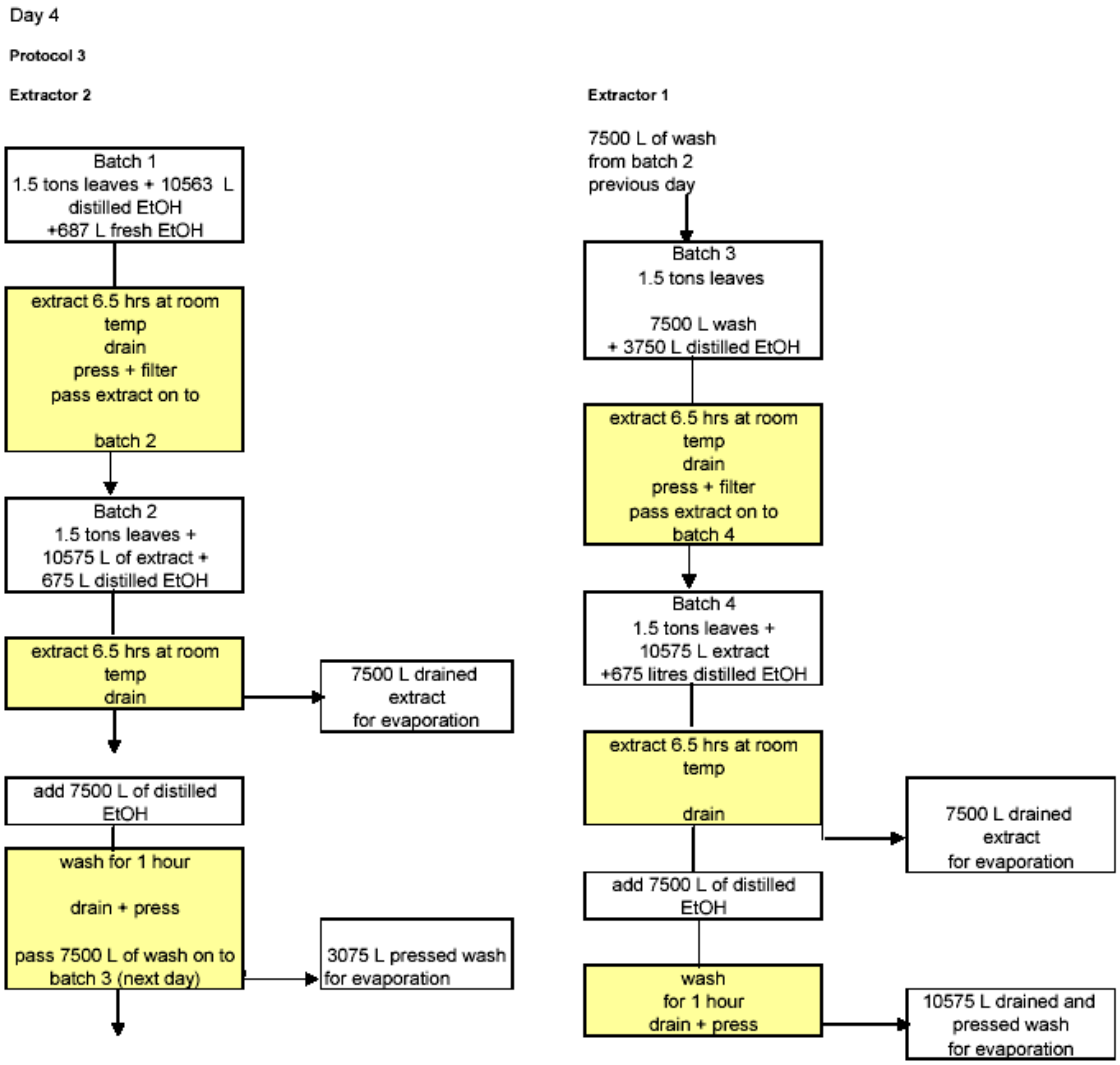
Capacity requirements for major elements:

1. Extractor: 2 Extractors, 12,000 litres each, each capable of handling 1.5 tons of leaves per batch

2. Evaporator and Rectification column: 1450 litres per hour

3. Press: 1500 litres spent leaves per hour

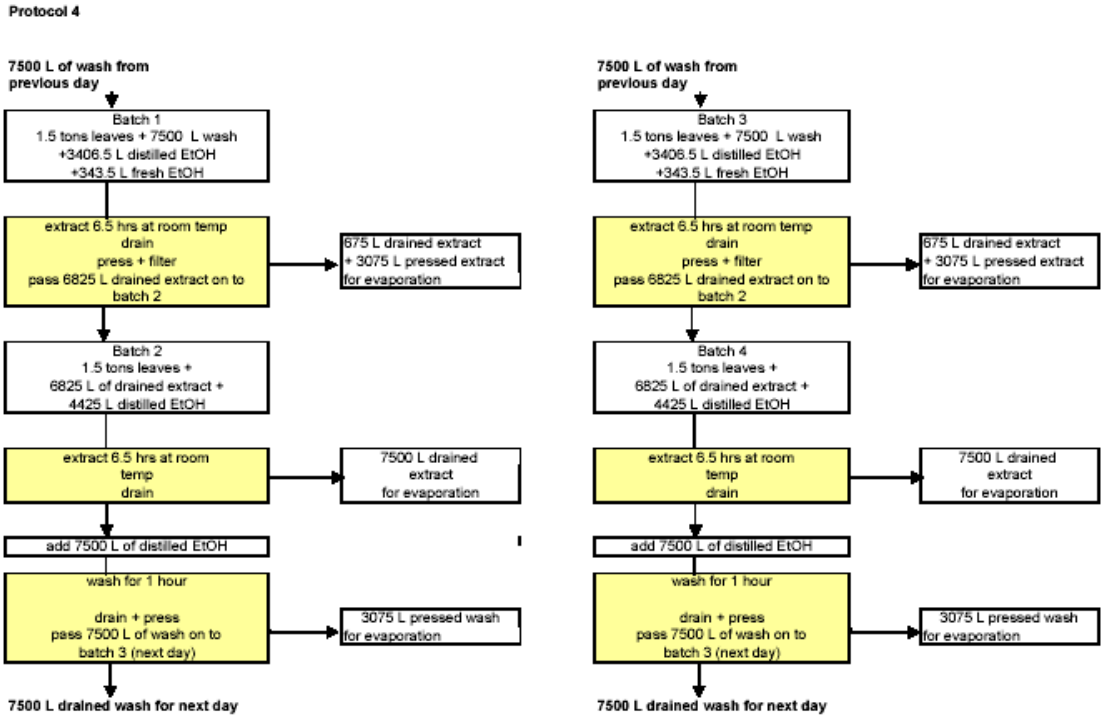
Annex No 4 : Process flow diagram



Continuous Flow

For a facility working on a commercial scale, a four batch protocol would be too complicated and returning the extract collected from the press after the extraction of the first and the third batch would also be inconvenient. A continuous two- batch protocol where an equilibrium has been established so that the drained wash of the second batch can continuously be used on the next first batch would be preferable. It would, however, take a larger series of trials to establish such an equilibrium. Mass balances for this protocol 4 would remain approximately the same as for protocol 3.

The two extractors would operate in the following manner:



Annex No 5: Mass balance sheet

Explanations: mass balance sheet

1. Procedures:

1.1 Protocol 3
Duration of extraction is 6.5 hrs
Batch 1 : extract, drain, press; pass drained and pressed extract on to batch 2
Batch 2 : extract (using extract from batch 1 + balance of additional EtOH), drain; pass extract on to evaporation, wash (with new EtOH), drain; pass drained wash on to batch 3, press; pass remaining wash to evaporation.
Batch 3: extract (using wash drained from Batch 2 plus additional EtOH), drain, press; pass drained and pressed extract on to batch 4
Batch 4 : extract (using extract of batch 3 plus additional EtOH), drain (pass extract on to evaporation), wash (with new EtOH), press, pass pressed extract on to evaporation.

1.2 Handling of liquids:
There are two tanks collecting extract and wash destined for evaporation (1 for each extractor).
All harvested extract from each day is pooled and evaporated the next day (alternating tanks)
Each extractor has an additional separate tank for extract or wash to be reused on the next batch.

1.3 Establishing equilibrium with two extractors:
On the 1st day, only extractor 1 is used, on the 2nd day both extractors work, extractor 1 continues with batch 3+4, extractor 2 starts with 1+2, so all four batches run on the same day. Extract +wash pooled from each day have same content.

2. Data regarding the process

2.1 Total amount of leaves to liquid in extractor during extraction: 1 kg : 7.5 litres or (1.5 tons 11.25 K litres, for 6 tons 45 K litres)
2.2 Amount of extract that can be collected by draining without pressing:
per kg of fresh leaves: 1: 5.0 , amount that remains on leaves after draining 1: 2.5 (for 1.5 tons 7.5 K litres harvested , for 1.5 tons 3.75 K litres remain on leaves after draining)
2.3 Volume of spent leaves sent for pressing per 1.5 tons of fresh leaves: 7500 litres
2.4 Volume of extract recovered by pressing:
The volume of extract recovered by pressing from 1.5 tons of leaves is 3075 litres , extract that remains entrained is 675 litres (0.45 litres per kg of fresh leaves)
2.5 Volume of spent pressed leaves sent for toasting per 1.5 tons of fresh leaves: 5000 litres
2.6.Volume of ethanol recovered by toasting per 1.5 tons of fresh leaves : 600 litres
2.7 Volume of ethanol not recovered on leaves per 1.5 tons of leaves : 75 litres
2.8 Amount of extract plus wash sent for evaporation after running batch 1,2,3,4 : = 28850 litres
2.9 Concentration of extract prior to purification: 1: 73, followed by drying

Mass balance sheet

Mass balance Protocol 3

day 1	input	input	input	circulation	circulation	output	output	output	output	hours
extraction	dried leaves, tons	new ethanol used litres	distilled ethanol used litres	drained extract reused same day	drained wash reused same day	drained extract reused next day	drained wash reused next day	drained extract or wash sent to evaporation	output drained leaves litres	
	3	19426	0	7500	0	0	7500	7500	15000	19
	input	input	input	circulation	output	output	output	hours		
pressing	drained leaves litres	input litres of extract entrained on drained leaves	litres of wash entrained on drained leaves	litres extract sent back to extractor	litres of extract or wash sent for eva-poration	pressed leaves litres	litres of extract or wash entrained on pressed leaves			
	15000	7500	3750	3075	3075	10000	1350	10		
	input	output	input	output	output	hours				
toasting	pressed leaves litres	toasted leaves	input ethanol on pressed leaves	output distilled ethanol recovered	output ethanol not recovered					
	5000	5000	675	600	75	5				
day 2	input	input	input	circulation	input	output	output			
extraction	dried leaves, tons	new ethanol used litres	distilled ethanol used litres	circulation drained extract reused same day	drained wash of previous day reused	drained wash reused next day	output drained extract or wash sent to evaporation	output drained leaves litres	hours	
	6	30750	600	15000	7500	7500	22500	30000	19	
	input	input	input	circulation	output	output	output	hours		
pressing	drained leaves litres	litres of extract entrained on drained leaves	litres of wash entrained on drained leaves	litres of pressed extract sent back to extractor	litres of extract or wash sent for eva-poration	litres pressed leaves	litres of extract or wash entrained on pressed leaves			
	30000	7500	7500	6150	6150	10000	2700	20		
	input	output	output	hours						
evaporation	extract or wash drained or pressed	distilled EtOH	concentrated extract (1/73)							
	10575	10432	143	20						
	input	output	input	output	output					
toasting	pressed leaves litres	toasted leaves	ethanol on pressed leaves	ethanol recovered	ethanol not recovered	hours				
	15000	15000	2025	1800	225	15				
day 3	input	input	input	circulation	input	output	output	output		
extraction	dried leaves, tons	new ethanol used litres	distilled ethanol used litres	circulation drained extract reused same day	drained wash of previous day reused	drained wash reused next day	output drained extract or wash sent to evaporation	drained leaves litres	hours	
	6	19118	12232	15000	7500	7500	22500	30000	19	
	input	input	input	circulation	output	output	output	output		
pressing	drained leaves litres	litres of extract entrained on drained leaves	litres of wash entrained on drained leaves	litres of pressed extract sent back to extractor	litres of pressed wash sent for eva-poration	litres pressed leaves	litres of extract or wash entrained on pressed leaves	hours		
	30000	7500	7500	6150	6150	10000	2700	20		
	input	output	output	hours						
evaporation	extract or wash drained or pressed	distilled EtOH	concentrated extract (1/73)							
	28650	28263	387	20						
	input	output	input	output	output					
toasting	pressed leaves litres	toasted leaves	ethanol on pressed leaves	ethanol recovered	ethanol not recovered	hours				
	20000	20000	2700	2400	300	20				

day 4									
extraction	input	input	input	circulation	input	output	output	output	hours
	dried leaves, tons	new ethanol used litres	distilled ethanol used litres	circulation drained extract reused same day	drained wash of previous day reused	drained wash reused next day	drained extract or wash sent to evaporation	drained leaves litres	
	6	687	30663	15000	7500	7500	22500	30000	19
pressing	input	input	input	circulation	output	output	output	hours	
	drained leaves litres	litres of extract entrained on drained leaves	litres of wash entrained on drained leaves	litres of pressed extract sent back to extractor	litres of extract or wash sent for eva-poration	litres pressed leaves	litres of extract or wash entrained on pressed leaves		
	30000	7500	7500	6150	6150	10000	2700	20	

evaporation	input	output	output	hours
	extract or wash drained or pressed litres	distilled EtOH	concentrated extract (1/73)	
	28650	28263	387	20

drying of extract	output
	dried extract kg
	340

toasting	input	output	input	output	output	hours
	pressed leaves litres	toasted leaves	ethanol on pressed leaves	ethanol recovered	ethanol not recovered	
	20000	20000	2700	2400	300	20

total amount of liquid used per 6 tons/day	ratio leaves/liquid	
31350	1	5.23

basic loading	fresh ETOH used	minus daily re-placement
day 1	19426	18739
day 2	30750	30063
day 3	19118	18431
basic loading		67,233

EtOH replacement		
%	daily litres	(250 days)
2	687	171,694
evaporation /rectification	28,650	7,162,500

Annex No 6: Estimates of economic feasibility

Introductory remarks

1. In order to analyse the economic feasibility in an African country, Tanzania was used as the example. Scales for salaries and wages were those of 2006. For diesel, the price of summer 2007 was used: 1 USD per litre. Ethanol (96%) was also calculated at 1 USD per litre including transport on the assumption that negotiations with the cheapest provider would prove successful. Electricity was calculated at 0.08 USD per kWhr. No costs were calculated for water (for the steam), assuming the facility has a suitable well.

2. Storage of the harvest was supposed to take place at the level of the agricultural enterprise – as is current practice - with only a ten day supply at the enterprise and a small buffer stock in the vicinity.

3. In the absence of a customised purification protocol the costs and yields of purification were estimated from the protocol given by Rodrigues et al.

4. The extraction facility was treated as a separate entity without any agricultural activity of its own, receiving dried leaves at factory gate prices that can currently be observed in East Africa. It was assumed that the agricultural entrepreneurs involved could look after themselves, and meet all the costs except those for the initial agricultural extension (training of staff and out-growers in the new cultivation techniques) **.** It was estimated that for the first two years about 150,000 USD would be needed per year, in the third year 70,000 USD might be needed and thereafter the costs would decrease further. Under optimal circumstances at least these costs, which are real development costs, might be borne by some development agency.

5. As far as capital costs are concerned, it was assumed that the relation between equity and loan would be 50/50 and that a loan with an interest rate of 7% and a seven year repayment period including one year of grace could be procured.

6. The assumption was made that during the first year of operation, at least 2/3 of the necessary leaves would already be available and that in the second year the facility would be able to operate at full capacity. As any sensitivity analysis would show, the availability of sufficient leaves of relatively high artemisinin content is the biggest risk to the profitability of the enterprise, hence the great importance of the extension efforts. The type of plant species which is used should have an artemisinin content of 1%, and some farmers, though by no means all, currently regularly achieve over 95%. A second calculation was made for raw material which has only 0.85% artemisinin content.

7. The price of artemisinin was calculated to allow for a profit of about 15% before tax (after meeting all capital expenses, repayment of the loan and deductions for depreciation). This should be sufficiently attractive to potential investors.

ethanolic extraction economic calculations

			Standard Raw Material	Standard Raw Material	Standard Raw Material
Positions	units		US Dollar	US Dollar	US Dollar
basic data			2/3 capacity	full capacity	full capacity
			year 1	year 2	year 3
rate of exchange	€/USD		1.36	1.36	1.36
quality of raw mat content art	0.95%				
extraction rate	93.00%				
purification rate	80.00%				
% purif. art extracted from leaves			0.71%	0.71%	0.71%
price of raw mat	US$/kg		0.70	0.70	0.70
yield of raw mat	ton/hectare		2.00	2.00	2.00
Data of production					
input leaves	kg/hr		250.00	250.00	250.00
daily production	hr/day		18	24	24
annual production	days/year		250.00	250.00	250.00
processed tons raw material	per year		1,125.00	1,500.00	1,500.00
ha planted			563	750	750
proceeds					
Sales Price Artesunat / kg	US$/kg		548.00	543.00	531.00
costs of derivatisation	USD/kg		220.00	220.00	220.00
sales price Artemisinine / kg	US$/kg		328.00	323.00	311.00
volume artemisinin	kg		7,951.50	10,602.00	10,602.00
sum proceeds			2,608,092.00	3,424,446.00	3,297,222.00
raw material costs			**787,500.00**	**1,050,000.00**	**1,050,000.00**
Processing costs	**USD per day**				
electrity	165.65		27,608.33	41,412.50	250.00
evaporation	210.80		35,133.33	52,700.00	52,700.00
rectification	47.87		7,978.33	11,967.50	11,967.50
solvent replacement	687.00		114,500.00	171,750.00	171,750.00
purification costs			40,000.00	60,000.00	60,000.00
sum processing costs			**225,220.00**	**337,830.00**	**296,667.50**
wages	**USD monthly**	**per shift**			
Managing Director			50,000.00	50,000.00	50,000.00
Manager finance			18,018.00	18,018.00	18,018.00
secretary			4,805.00	4,805.00	4,805.00
accountant			7,580.00	7,580.00	7,580.00
watchman			1,800.00	1,800.00	1,800.00
Mechanic/control+maintenance	2000		96,000.00	48,000.00	48,000.00
2 chemists	2000		24,000.00	24,000.00	24,000.00
agric co-ordinator	2500		30,000.00	30,000.00	30,000.00
supervisor	2000	1	48,000.00	72,000.00	72,000.00
technician/production	1500	2	72,000.00	108,000.00	108,000.00
production workers	600	4	57,600.00	86,400.00	86,400.00
helpers	250	2	12,000.00	18,000.00	18,000.00
drivers	700	2	33,600.00	50,400.00	50,400.00
Sum Personell			455,403.00	519,003.00	519,003.00
25% Payrolltax			113,850.75	129,750.75	129,750.75
sum personell costs			**569,253.75**	**648,753.75**	**648,753.75**
other costs					
administration , communication			30,000.00	30,000.00	30,000.00
rent for godown			18,000.00	18,000.00	18,000.00
maintenance			3,000.00	50,000.00	70,000.00
agricultural extension			150,000.00	150,000.00	70,000.00
sum other costs			**201,000.00**	**248,000.00**	**188,000.00**
sum production costs			1,782,973.75	2,284,583.75	2,183,421.25
interest on 1/2 prod costs	15%		133,723.03	171,343.78	171,343.78
production costs + interest overdrafi			**1,916,696.78**	**2,455,927.53**	**2,354,765.03**
investments	€	in US$			
complete extraction unit	1,800,000.00		2,448,000.00	2,448,000.00	2,448,000.00
purification/crystallization unit	100,000.00		136,000.00	136,000.00	136,000.00
contingency	200,000.00		272,000.00	272,000.00	272,000.00
basic loading solvent for extraction		1,00 p Litre	44,822.00	22,411.00	0.00
basic loading solvent for purification	3,000.00		906.67	1,360.00	0.00
mobile equipment+lab		p squ m	200,000.00	200,000.00	200,000.00
building/Produktion 200 sqm)		500.00	100,000.00	100,000.00	100,000.00
Building/ office/lab(200 sqm)		400.00	80,000.00	80,000.00	80,000.00
Building /store (200 sqm)		300.00	60,000.00	60,000.00	60,000.00
Sum Investments			**3,341,728.67**	**3,319,771.00**	**3,296,000.00**
capital costs	year				
depreciation plant	10		305,600.00	305,600.00	305,600.00
depreciation buildings	20		12,000.00	12,000.00	12,000.00
interest Loan/supplier credit	7%		116,191.99	116,191.99	96,826.65
repayment loan/supplier credt	6 years		0.00	276,647.58	276,647.58
sum capital costs			**433,791.99**	**710,439.57**	**691,074.24**
sum all costs			**2,350,488.77**	**3,166,367.10**	**3,045,839.27**
proceeds minus costs			**257,603.23**	**258,078.90**	**251,382.73**
equity	50.00%		1,659,885.50	1,659,885.50	1,659,885.50
loan	50.00%		1,659,885.50	1,659,885.50	1,383,237.92
annual return (minus depreciation, minus interest,minus repayment of loan) to equity before tax		%	15.52%	15.55%	15.14%

Table: Economic calculations

ethanolic extraction economic calculations

			Inferior Raw Material	Inferior Raw Material	Inferior Raw Material
Positions	units		US Dollar	US Dollar	US Dollar
basic data			2/3 capacity	full capacity	full capacity
			year 1	year 2	year 3
rate of exchange	€/USD		1.36	1.36	1.36
quality of raw mat content art	0.85%				
extraction rate	93.00%				
purification rate	80.00%				
% purif. art extracted from leaves			0.63%	0.63%	0.63%
price of raw mat	US$/kg		0.55	0.55	0.55
yield of raw mat	ton/hectare		2.00	2.00	2.00
Data of production					
input leaves	kg/hr		250.00	250.00	250.00
daily production	hr/day		18	24	24
annual production	days/year		250.00	250.00	250.00
processed tons raw material	per year		1,125.00	1,500.00	1,500.00
ha planted			563	750	750
proceeds					
Sales Price Artesunat / kg	US$/kg		560.00	555.00	542.00
costs of derivatisation	USD/kg		220.00	220.00	220.00
sales price Artemisinine / kg	US$/kg		340.00	335.00	322.00
volume artemisinin	kg		7,114.50	9,486.00	9,486.00
sum proceeds			2,418,930.00	3,177,810.00	3,054,492.00
raw material costs			**618,750.00**	**825,000.00**	**825,000.00**
Processing costs	**USD per day**				
electrity	165.65		27,608.33	41,412.50	250.00
evaporation	210.80		35,133.33	52,700.00	52,700.00
rectification	47.87		7,978.33	11,967.50	11,967.50
solvent replacement	687.00		114,500.00	171,750.00	171,750.00
purification costs			40,000.00	60,000.00	60,000.00
sum processing costs			**225,220.00**	**337,830.00**	**296,667.50**
wages	**USD monthly**	**per shift**			
Managing Director			50,000.00	50,000.00	50,000.00
Manager finance			18,018.00	18,018.00	18,018.00
secretary			4,805.00	4,805.00	4,805.00
accountant			7,580.00	7,580.00	7,580.00
watchman			1,800.00	1,800.00	1,800.00
Mechanic/control+maintenance	2000		96,000.00	48,000.00	48,000.00
2 chemists	2000		24,000.00	24,000.00	24,000.00
agric co-ordinator	2500		30,000.00	30,000.00	30,000.00
supervisor	2000	1	48,000.00	72,000.00	72,000.00
technician/production	1500	2	72,000.00	108,000.00	108,000.00
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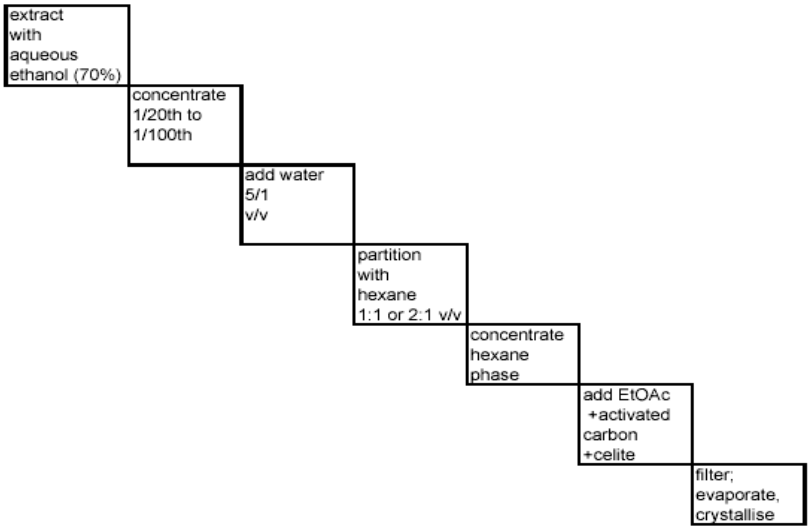
25% Payrolltax			113,850.75	129,750.75	129,750.75
sum personell costs			**569,253.75**	**648,753.75**	**648,753.75**
other costs					
administration , communication			30,000.00	30,000.00	30,000.00
rent for godown			18,000.00	18,000.00	18,000.00
maintenance			3,000.00	50,000.00	70,000.00
agricultural extension			150,000.00	150,000.00	70,000.00
sum other costs			**201,000.00**	**248,000.00**	**188,000.00**
sum production costs			1,614,223.75	2,059,583.75	1,958,421.25
interest on 1/2 prod costs	15%		121,066.78	154,468.78	154,468.78
production costs + interest overdraft			**1,735,290.53**	**2,214,052.53**	**2,112,890.03**
investments	€	in US$			
complete extraction unit	1,800,000.00		2,448,000.00	2,448,000.00	2,448,000.00
purification/crystallization unit	100,000.00		136,000.00	136,000.00	136,000.00
contingency	200,000.00		272,000.00	272,000.00	272,000.00
basic loading solvent for extraction		1.00 p Litre	44,822.00	22,411.00	0.00
basic loading solvent for purification	3,000.00		906.67	1,360.00	0.00
mobile equipment+lab		p squ m	200,000.00	200,000.00	200,000.00
building/Produktion 200 sqm)		500.00	100,000.00	100,000.00	100,000.00
Building/ office/lab(200 sqm)		400.00	80,000.00	80,000.00	80,000.00
Building /store (200 sqm)		300.00	60,000.00	60,000.00	60,000.00
Sum Investments			**3,341,728.67**	**3,319,771.00**	**3,296,000.00**
capital costs	year				
depreciation plant	10		305,600.00	305,600.00	305,600.00
depreciation buildings	20		12,000.00	12,000.00	12,000.00
interest Loan/supplier credit	7%		116,191.99	116,191.99	96,826.65
repayment loan/supplier credt	6 years		0.00	276,647.58	276,647.58
sum capital costs			**433,791.99**	**710,439.57**	**691,074.24**
sum all costs			**2,169,082.52**	**2,924,492.10**	**2,803,964.27**
proceeds minus costs			**249,847.48**	**253,317.90**	**250,527.73**
equity	50.00%		1,659,885.50	1,659,885.50	1,659,885.50
loan	50.00%		1,659,885.50	1,659,885.50	1,383,237.92
annual return (minus depreciation, minus interest,minus repayment of loan) to equity before tax		%	15.05%	15.26%	15.09%

Table: economic calculations

Annex No 7: Methods of purification of ethanolic extracts

The most simple procedure for purifying ethanolic extracts without the use of chromatography and without employing any particularly toxic solvent has been suggested by Kumar at al [28]
Their protocol can be summarised as follows:



Kumar el al do not give any figure for the content of the leaves they used and thus nothing can be said regarding the efficiency of this operation. This protocol could, however, not be reproduced with extract from the study reported here: While partitioning the crude extract with hexane and water removed a larger part of the polar impurities, further treatment with activated carbon in an ethyl acetate/hexane solution did not produce a sufficiently enriched extract that would crystallise.

Ferreira Rodrigues et al [29] developed a specific multistage extraction procedure for the ethanolic extraction of ethanol, which allowed for an extraction rate of 91.3%. The purification procedure they used is fairly complicated :

"the (filtered ethanolic)extracts were mixed, concentrated to 30% of the initial volume and treated with the activated carbon Carbomafra (Curitiba, Brazil) for 1.5 hours under mechanical agitation at room temperature. The carbon was eliminated through vacuum filtration using Büchner funnels with a precoat of diatomaceous earth (Celite). A solution of a dark yellow colour was the result. It was evaporated until it became dry, in order to determine the outcome of the extraction. Ethanol and industrial silicon dioxide of the type Zeosil 175, Rhodia (Paulínia, Brazil) were added to the dry extract in proportion 1:1 (dry extract: Zeosil). The silica and the extract were mixed with a stirrer of the type Munson (New York, USA). The ethanol subsequently vanished by distillation in vacuum, which was achieved by a rotary evaporator of the type Büchi, model R-151 (Flawil, Switzerland). The mixture was added to the top of a column of stainless steel previously packed with the same stationary phase, for the chromatographic separation by filtration through a column under vacuum… During the column chromatography stage, gradient elution was initiated with hexane permitting a separation of fats and other non-polar components, making subsequent enrichment of the artemisinin possible in the mobile hexane/ ethyl acetate phase…

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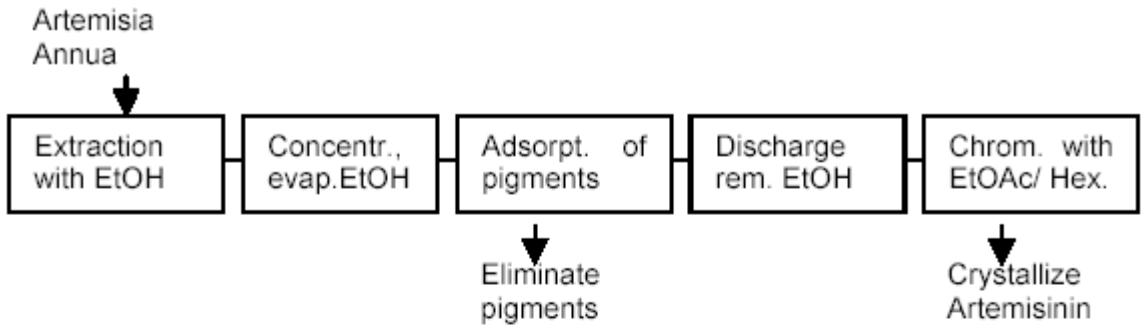
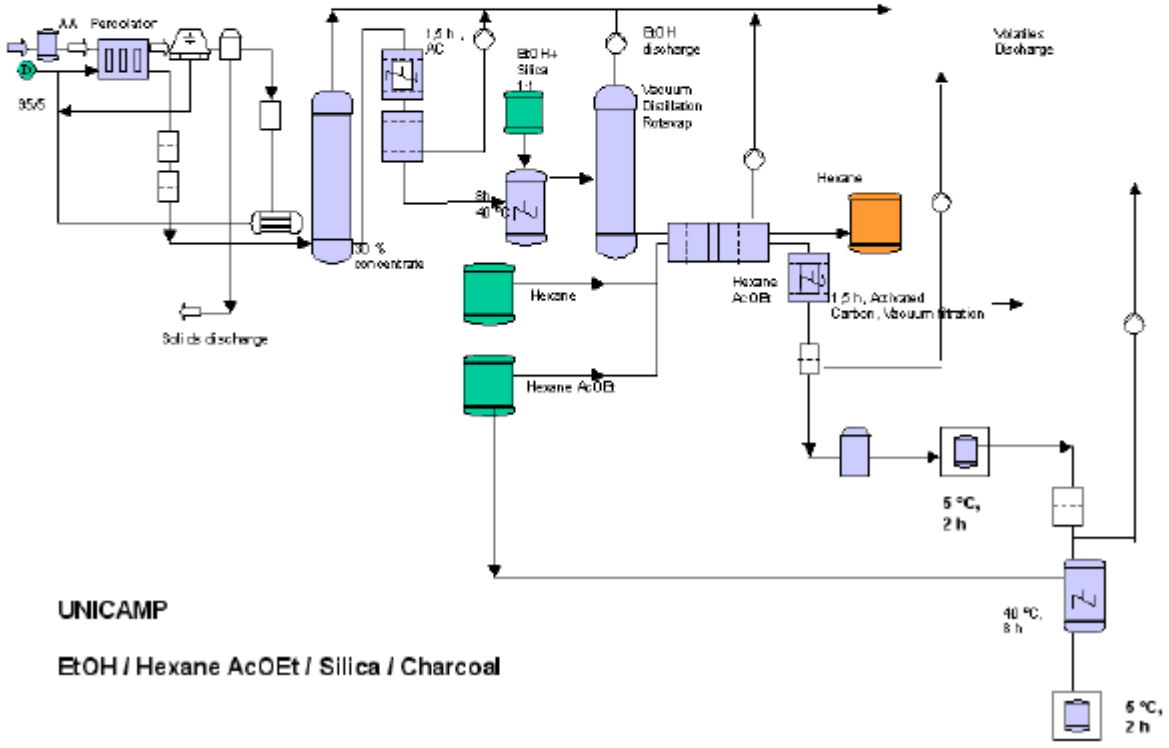
28 Kumar Sushil; Gupta Shiv Kumar; Singh Digvijay ; Gupta Madan Mohan; Jain Dharam Chand; Kahol Atul Prakash ; Khanuja Suman Preet Singh; Ram Govind : Process for isolating artemisinin from artemisia annua; US patent 6685972, 2003-10-02

29 Rodney Alexandre Ferreira Rodrigues†, Mary Ann Foglio, Sinésio Boaventura Júnior, Adriana da Silva Santos and Vera Lúcia Garcia Rehder: Optimization of the Extraction and Isolation of the Antimalarial Drug Artemisininin from *Artemisia annua* L.**;** Multidisciplinary Centre of Chemical Research, Biology and Agriculture, State University of Campinas
CP 6171, 13081-970 Campinas – SP, Brazil
Received on 30 September 2004; accepted on 27 July 2005; published on the web on 20 January 2006

The enriched part was treated with activated carbon under agitation for half an hour at room temperature, and subsequently filtered in vacuum. A yellowish solution was the outcome. It was evaporated down to 5% of the initial volume, and cooled at 5°C for two hours in order to crystallize the active substance. The crystals were filtered in vacuum through filter paper and a Büchner funnel, and washed with a mixture of hexane and ethyl acetate (85/15, v/v) that had been previously cooled down to 5°C." [30]

The process can be illustrated as follows [31]:





This protocol does involve chromatography, but since they used Zeosil rather than silica gel, the process is cheaper than in other protocols and possible problems due to acidity of silica gel can also be avoided.

80% of the artemisinin available in the extract could be recovered during purification.

[30] Loc cit, translation from Brazilian

[31] GFA consultancy group: Report for Kreditanstalt für Wiederaufbau, unpublished manuscript, Frankfurt/main 2006

While care was taken to use relatively cheap inputs, they were all treated as consumables in the economic calculations of this purification study. Recycling of most of the inputs used is possible.

Peter Griffee has reported extractions with ethanol being followed by a second extraction process with gasoline and benzene followed by crystallisation with ethanol. Use of benzene is, however, not acceptable because of the health hazards associated with it.

Ethanol extraction followed by extraction with supercritical or sub-critical CO2 has been tried successfully on several botanical extracts including artemisia [32]. However, no details of the protocol used were published. It is possible to extract artemisinin from artemisia with ScCO2 [33], using ethanol as an entrainer (of 2-10% w/w). Very high extraction rates with this technology are reported. It can therefore be expected that purification with ScCO2 should also be successful. High capital and operational costs may, however, turn out to be an obstacle to the use of this technology[34]. Since extraction of artemisinin is generally more successful at lower temperatures it may well be that sub-critical CO2 may be more effective. Capital expenditure and running costs would be slightly lower in this case. On the other hand recycling of the solvent is said to be more difficult. Presumably some kind of final purification will also be necessary after the treatment with CO^2.

Another possibility might be the use of hydrofluorocarbons. The use of 1,1,1,2-tetrafluoroethane (hydrofluorocarbon R134a) has been recommended for the extraction of artemisinin from artemisia[35] . There is, however, no proof that the extraction rate that can be achieved in the extraction of the leaves would warrant the higher capital expenditure associated with this technology. Preliminary trials done in the context of this study suggest that HFC R134 a might, instead, be used to purify ethanolic extracts: If crude extract is dried and extracted with HFC R134a, a semi-refined extract containing about 60% artemisinin is harvested, which is amenable to crystallisation[36]. The extraction efficiency of this process would have to be optimised

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32 *FAIR-CT96-2003*
EXTEN: Volume extraction and encapsulation of substances used as flavour chemicals, pharmaceutical raw substances, biochemicals and enzymatic systems

33 Studies on extraction of artemisinin from *Artemisia annua* by supercritical carbon dioxide. He, Chunmao; Liang, Zhongyun. Res. Inst. Chem. Process. Utilisation Forest Products, Guangsi Acad. Forestry, Nanning. Zhongcaoyao (199), 30(7), 497-499.
Abstract: *A. annua* was extracted by supercritical carbon dioxide at different pressures, temperatures and contact time to optimise the yield (>95%) of artemisinin. The carbon dioxide extract was purified by other separation methods to obtain a final product with a purity over 99%. The authenticity of the product was verified by TLC, IR, MS, 1HNMR and 13

Extraction of artemisinin and artemisinic acid from Artemisia annual L. using supercritical carbon dioxide. Kohler M, Haerdi W, Christen P, Veuthey JL:; J. Chromatogr. A. 1997 Oct 17;785(1-2), pp.353-60.
Abstract: Artemisinin and its major precursor artemisinin acid, isolated as the active principles of the medicinal plant *A. annua*, were extracted by supercritical fluid extraction and analysed by supercritical fluid chromatography using a capillary column, coupled with a flame ionisation detector. With optimised operating conditions, artemisinin and artemisinic acid were quantitatively extracted in less than 20 min. The supercritical fluid was composed of carbon dioxide and 3% methanol at 50 degrees C and 15 MPa. In all cases, artemisinin and artemisinic acid were extracted in a higher yield with supercritical carbon dioxide than with liquid solvent extraction processes.

34 See EXTEN study : BioMatNet database: European Union, *-FAIR-CT96-2003*

35 Cutler Malcolm, Rifkin Alexei; Comparative Assessment of Technologies for Extraction of Artemisinin, A summary of report commissioned through Malaria Medicines Ventures (MMV), August 2006

36 results from Dr. Detlef Freitag (Erlangen) and Dr Schuehly (Graz)

and to find the best method to clean the impure crystals still have to be determined. Since only a small HFC extractor would be needed in purification, capital costs would not be an issue.

Further research into purification techniques is needed.

Annex No 8 : Acknowledgements

In the study that has been reported above, the following people and institutions were involved:

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