Process Study of Anti-Malaria Product from Artemisia Annua

Seid Yimer, Omprakash Sahu[*]

Department of Chemical Engineering, KIOT, Wollo University, Ethiopia
*Corresponding author: ops0121@gmail.com

Abstract Malaria disease is endemic in least developed countries like Ethiopia. This disease is caused by protozoa of the genus Plasmodium infection that is easily recognizable from the symptoms of prolonged fever. The rapid development of drug-resistant malaria parasite strain leaves the need for new effective anti-malarial drugs. Artemisinin is a sesquiterpene lactone found in the leaves and flowers of plants Artemisia annua L and have different chemical structures and higher efficacy than others. The plant Artemisia annua is the sole source of artemisinin in the industry. The main objective of the project was to provide a simple, rapid, cost effective, environmental friendly and practical method for the isolation of artemisinin from the plant Artemisia annua Furthermore, this work was focus on the mass and energy balance calculation of the antibiotic Artemisia annua in the large scale industry.

Keywords: *Artemisia annua L., extraction, feasibility, hexane, malaria, sesquiterpene lactone*

Cite This Article: Seid Yimer, and Omprakash Sahu, "Process Study of Anti-Malaria Product from Artemisia Annua." *World Journal of Nutrition and Health*, vol. 2, no. 1 (2014): 10-16. doi: 10.12691/jnh-2-1-2.

1. Introduction

Artemisia has been used by Chinese herbalists for more than a thousand years in the treatment of many illnesses like skin diseases and malaria. Its anti-malarial application was first described in Zhouhou Beji Fang edited in the middle of the fourth century by Ge Hong [1]. In the 1960s a research program was set up by the Chinese army to find an adequate treatment for malaria [2]. In the early 1970s Chinese scientists screening traditional Chinese medicinal herbs for searching new anti-malarial isolated artemisinin from the herb known as Qing Hao (*Artemisia annua* L.) [3]. Artemisia annua is a common herb and has been found in many parts of the world. Encouraged by the pharmaceutical industry, plantations spread all over the world (Vietnam: 1970, Madagascar: 1993, Switzerland: 1991, East Africa: 1994, Australia - Papua Guinea: 1997-99, USA: 1999, Brazil - Argentina: 2002 -2007, West-Africa: 2005, UK: 2007). Today, Artemisia annua plantations are widespread in several African countries such as Gambia, Cameroon, DRC, Madagascar, Tanzania and Ethiopia [4,5].

The increased demand for artemisinin in Africa starting in 2004 stimulated the efforts to increase the production of Artemisia in East Africa. ABE clearly was in a position to do so. The area subsequently placed under various production arrangements. The planted area was in Kenya (nearly 65%) followed by Uganda (19%) and Tanzania (north, over 16%). Both small and large farms were involved. In 2006, 7,500 farmers were reportedly involved, but area was not revealed. Currently the plant is widely cultivating in many places of Ethiopia [6]. The chemical compositions of Artemisia annua are shown in Figure 1.
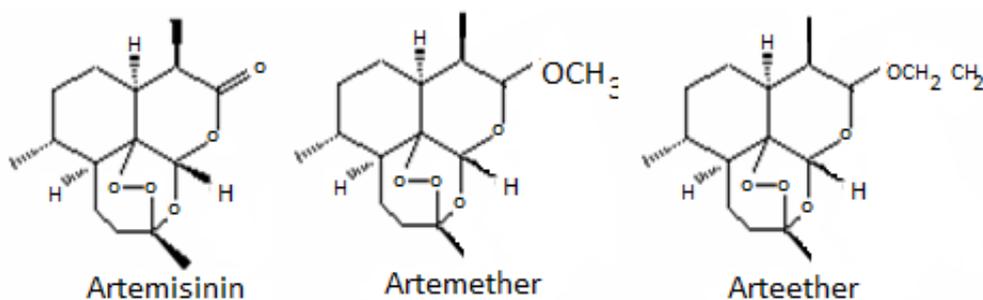

Figure 1. Chemical structure of Artemisia annua

Artemisia annua essential oil is beneficial for anti-bacteria, anti-periodic, anti-septic and anti-cancer properties. It is commonly used in tropical nations which can afford it, preferentially as part of a combination-

cocktail with other anti malarias in order to prevent the development of parasite resistance. Artemisinin is an unusual sessquiterpene lactone incorporating an endoperoxide group. It has potent anti-plasmodial activity and was shown in clinical trials to be highly effective against malaria, including patients with cerebral malaria and patients with malaria parasites resistant to chloroquine [7,8]. In contrast to other antimalarials such as quinine, (used for treating chloroquine-resistant malaria), artemisinin was found to be remarkably non-toxic and the importance of its discovery against the background of a rising incidence of multidrug-resistant malaria parasites cannot be underestimated. Several semi-synthetic artemisinin derivatives including artemether, arteether and sodium artesunate are now in clinical use worldwide for the treatment of malaria [9]. However, antimalarial drugs such as the artemisinin derivatives are expensive and are not accessible to the majority of people who are at risk of malaria. Malaria still kills close to one child every minute in Africa. Worldwide, it causes the death of around 10 000 pregnant women and 200 000 new born. In Africa, it is the third most deadly disease among children. It contributes heavily to teachers' and pupils school absenteeism and impairs their learning process and capacities. While poverty is the prime cause of school drop outs on the continent, poor health and nutrition could well prove, like in Northern countries, critical obstacles to quality education [10]. The plant has also been shown to have anti-cancer properties. It is said to have the ability to be selectively toxic to some breast cancer cells and some form of prostate cancer, there have been exciting preclinical results against leukemia, and other cancer cells. Artemisinin is the active component of the herb Artemesia annua.

2. Material and Method

2.1. Material

Botanically Artemisia annua is a vigorous weedy annual which is single-stemmed and ranges in height from one to two meters. It grows easily in temperate areas and tropical areas at higher altitudes and is raised in an increasing number of countries. It is well suited to both small-scale and plantation culture. The seed is extremely small and is usually grown to the seedling stage and transplanted [11]. Relatively few inputs are needed, aside from some fertilization, because the plants at present do not seem to have any particular insect or disease problems. Normally some water is required to establish the crop and dry weather is needed at the harvest and for drying. Artemisinin levels of the plants tend to vary by variety, but the influences of area and growing conditions are not yet clear. It is principally planted early in the calendar year and needs five to six months to mature [12,13]. The image of Artemisia annua is shown in Figure 2.

Figure 2. The plant Artemisia annua [6]

The leaf of Artemisia annua growth stage of five month was collected before the flowering of the plant, from Wondogenet (270 km south west of Addis Ababa), kombolcha and kalu (360-376 Km in north east of Ethiopia) and Gonder (724 Km north west of Ethioia) where it is cultivated for different research purposes[14]. The Wondogenet Artemesia annua leaves were obtained from essential oil research center of Ethiopia by pass all the steps of collection, drying and milling unit operations, but others were not passed the above unit operations. Finally we did the extraction and clean up of the crude extract in Bahir Dar University Chemical Engineering Department laboratory, Ethiopia.

2.2. Methods

The oil was extracted by hexane solvent and rotary evaporator was used for purification of the oil.

2.2.1. Extraction Appartus

The extractor consist a heating device, solvent reservoir round bottom flask, extraction chamber containing a porous paper extraction thimble and a condenser [15].

2.2.2. Experimental Steps

First 100g of Artemisia annua was placed in the thimble and covered with glass wool. It is placed in the extraction

chamber. After that we were adding an optimum ratio of 1: 7.5 of crushed Artemisia annua leaves with hexane solvent to maximize the oil product. After that the system was heating. After 5 hours, the solvent and extract in the extract flask was vaporized by rotary vapor to separate the oil from the solvent followed by artemisinin oil purification unit operation.

2.2.3. Large Scale Processing of Artemisinin

The collected Artemisia annua leaves are leave for sun drying, and12% of moisture is removed. Followed the leaves were crushed using miller in a size range of 3-5mm and the leaves entered to the extractor with optimum solvent ratio. The amount of dry biomass feed to the reactor depends on the capacity of extractor. In the extractor the best ratio of dry biomass to hexane is 1:7.5 (w/v). 20% of dry biomasses removed as spent leaves. This spent leaves will be used as a solid fuel and fertilizer after de-solventaized. In the de-solventaizer 10% of hexane recycled to the original tank and it takes 8 hrs. The crude extract which came from extractors went to solvent evaporator. In the evaporator 10% of the original amount of hexane recovered. In this section only the hexane is recycled and took 4hrs. The liquor from solvent

evaporators enters to the first crystallizer [16]. In this section 15% of the liquor removed as oils, waxes, hexane and 15% as precipitate wastes. This process is completed in 48hrs. Crystalline artemisinin from first crystallizer goes to wash tank and washing by 20 liters of hexane. In this section 70% is recycled to hexane tank and 10% of crystalline artemisinin removed as wax, oil and hexane and take 1hrs. On the second crystallizer 15% of crude artemisinin removed as wax, oil and hexane. In the crystallizer the crude artemisinin cool down to 5°C. In order to remove the greenish pigments of artemisinin we added color absorber charcoal. We used filtration to remove the impurities color. Then 15% of the crude artemisinin was removed as pigment and impurities. Small evaporator used after filtration and again 15% of color absorber is removed as pigments and impurities. Evaporation of final crystallization takes place in this process 1% impurity is removed. Amount of impurity is decrease by using successive evaporators. When we got satisfactory pure artemsinin content we stoped the extraction process. The process flow diagram for the production of antibiotic from the Artemisia annua is shown in Figure 3.
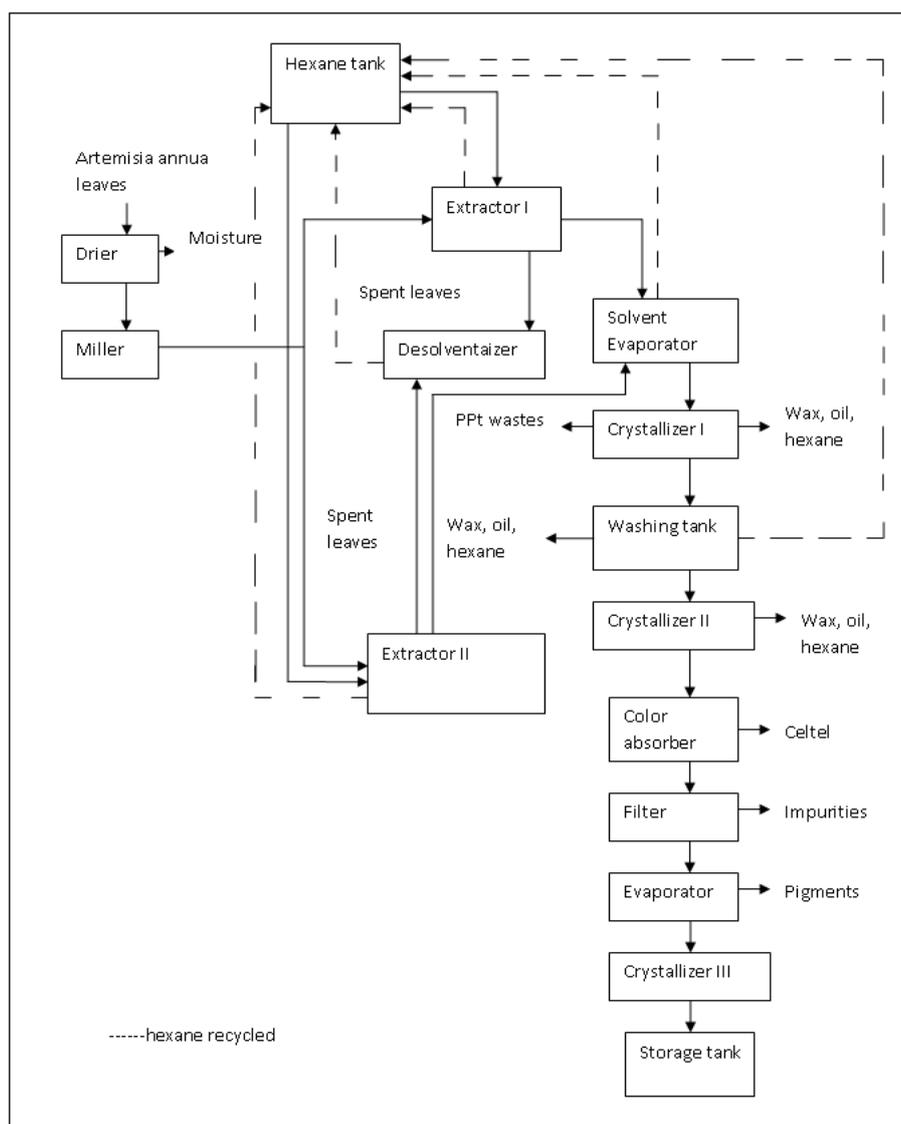

**Figure 3.** Antibiotics production from Artemisia annua

3. Result and Discussion

Our laboratory result showed us 1.2%, 0.95% and 0.5% artemisinin content oil was extracted from Wondo Genet, Kalu and Gonder Artemisia annua leaves respectively; which is similar result like the results of essential oils extraction center of Ethiopia from different parts of Ethiopia (0.1-1.4%) [17]. By using the laboratory result of wendogenet oil percentage and the assumed total output of artemisinin oil quantity we did mass and energy balance calculations for every unit operations accordingly by the flow sheet presented above.

3.1. Mass Balance Calculations

By taking yield of Artemisia annua leaves per annum in Ethiopia (in 2007 it was 426.1 Kg/h) as a reference all the material balances were done as follows for all unit operations.

3.1.1. Material Balance on Dryer
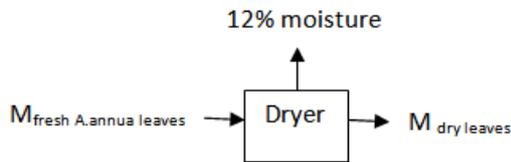
Assumption
- It is steady state process: accumulation = 0
- There is no chemical reaction (Mecanical unit operation): generation = consumption = 0

Input = Output

$M_{fresh\ A.annua\ leaves}$

$= 12\% * M_{fresh\ artemisia\ annua\ leaves}$

$+ M_{dry\ leaves}$

$426.1 \frac{Kg}{h} = 12\% * \frac{Kg}{h} + M_{dry\ leaves}$

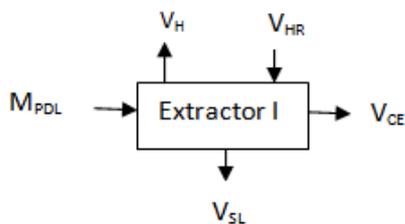$M_{dry\ leaves} = 374.97 \frac{Kg}{h}$

3.1.2. Material Balance on Miller

Assumptions are similar to the dryer because it is other kind of mechanical unit operation apparatus: Input= Output.

$M_{dry\ leaves} = M_{powereddry\ leaves} = 374.97 \frac{Kg}{h}$

3.1.3. Material Balance on Extracter I

Where;
M_{PDL} = Mass of powered dry leaves
V_{PDL} = Volumetric flow rate of dry leaves
V_H = Volumetric flow rate of hexane
V_{HR} = Volumetric flow rate of hexane recycle
V_{SL} = Volumetric flow rate of spent leaves
V_{CE} = Volumetric flow rate of crude extract

The optimum ratio of dry biomass to hexane is 1: 7.5. Before the mass balance calculation on the extractor unit similarity was performed. There are two equal extractors in the process a result of the process is continuous type. Consequently, at the cleaning and maintenance period at least one is continue the extraction process. So that the total mass flow rate is divided for these two extractors and changed to V_{PDL} by using density of Artemisia annua.

$$V_{PDL} = \frac{MPDL}{\rho} = \frac{187.48kg * 1000l * m^2}{1240kg * m^3 * h} = 151.19 \frac{1}{h}$$

$$V_H = 151.19 \frac{1}{h} * 7.5 = 1133.93 \frac{1}{h}$$

$$V_{PDL} + V_H = V_{HRE} + V_{SL} + V_{CE}$$

$$V_{CE} = 1141.51 \frac{1}{h}$$

3.1.4. Material Balance on Extractor II

Since the two extractors have equal size, identical input and output and similar mass flow rates material balance on extractor I is the same with extractor II. Therefore, the amount of crude extract and hexane used becomes double.

$V_{CE} = 2283 \frac{1}{h}$ and $V_H = 1133.93 \frac{1}{h}$.
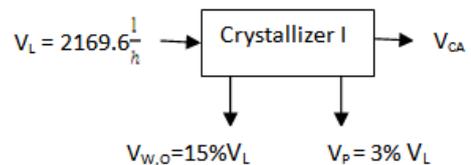
3.1.5. Material Balance on Solvent Evaporator
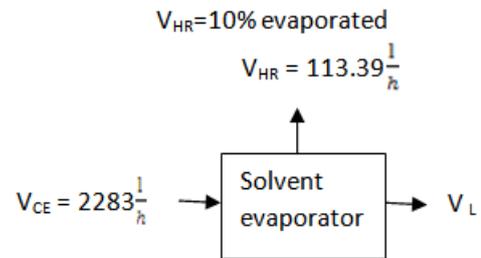
Where
V_L = volume of liquor
$V_{CE} = V_L + 10\% * V_{HRE}$

$VL = 2283 \frac{1}{h} - 113.39 \frac{1}{h} = 2169.6 \frac{1}{h}$
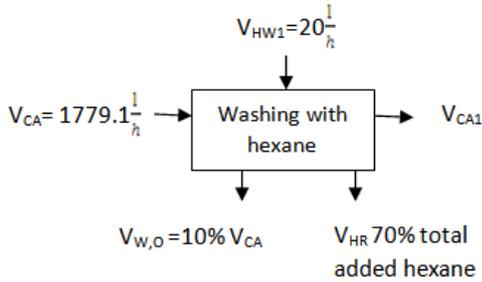
3.1.6. Material Balance on Crystallizer I

Where
$V_{W,O}$ = volume flow rate of waxes and oils
V_{CA} = volumetric flow rate of liquor crystalline artemisinin
V_P = volumetric flow rate of precipitate wastes

$$V_L = V_{W,O} + V_P + V_{CA}$$

$$V_{CA} = 2169\,\frac{1}{h} - (325.4 + 65.1)\,\frac{1}{h} = 1779.1\,\frac{1}{h}$$

3.1.7. Material Balance on Washer
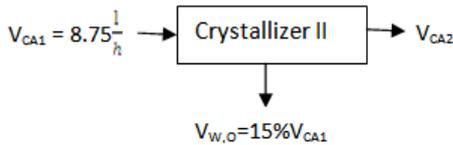

Where
V_{HW1} = volumetric flow rate of hexane washing from tank 1
V_{CA1} = volumetric flow rate of semi purified artemisinin 1

$$V_{CA} + V_{HW1} = 10\%\ V_{CA} + 70\%\ (V_{HW1} + V_{HW2}) + V_{CA1}$$

$$V_{CA1} = 8.75\,\frac{1}{h}$$

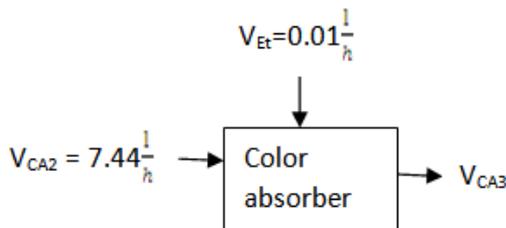### 3.1.8. Material Balance on Crystallizer II

Where
V_{CA2} = volumetric flow rate of semi purified artemisinin 2

$$V_{CA1} = V_{CA2} + 0.15 V_{CA1}$$

$$V_{CA2} = 7.44\,\frac{1}{h}$$

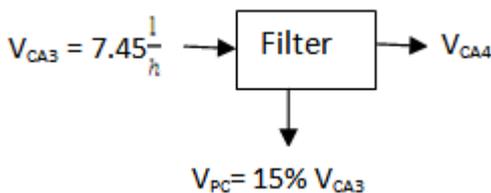### 3.1.9. Material Balance on Color Absorber

Where, V_{CA3} = volumetric flow rate of semi nn purified artemisinin 3
V_{Et} = volumetric flow rate of ethanoL

$$V_{CA2} + V_{Et} = V_{AC3}$$

$$V_{AC3} = 7.45\,\frac{1}{h}$$

3.1.10. Material Balance on Filter
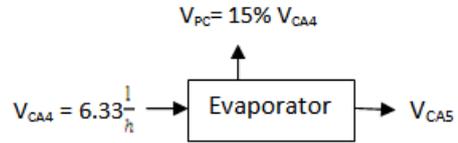

Where
V_{PC}= volumetric flow rate of pigments
V_{CA3}=volumetric flow rate of semi purified artemisinin 3

$$V_{CA3} = V_{AC4} + V_{PC}$$
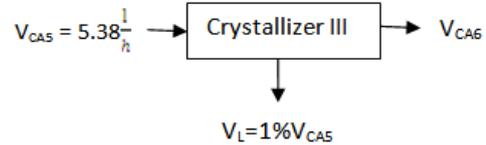
$$V_{AC4} = 6.33\,\frac{1}{h}$$

3.1.11. Material Balance on Small Evaporator

$$V_{AC4} = V_{PC} + V_{AC5}$$

$$V_{AC5} = 5.38\,\frac{1}{h}$$

3.1.12. Materiakl Baklance on Crystallizer III

V_{CA6} = volumetric flow rate of final Purified artemisinin

$$V_{AC5} = V_L + V_{CA6}$$

$$V_{CA6} = 5.33\,\frac{1}{h}$$

3.2. Energy BalanceCalculations

The required data to carry out energy balance calculation for the process is mentioned below.

- Mass flow rate of artemesia annua of dry leaves ($\dot{m}_A$)

$$= 187.48\,\frac{kg}{h}$$

- Volume flow rate of hexane (V_h) = $1133.9\,\frac{1}{h}$

- Density of hexane (ρ_h) = $660\,\frac{kg}{m^3}$

- Specific heat capacity of hexane (C_{ph}) = $2.26\,\frac{KJ}{Kg\,^\circ K}$

- Specific heat capacity of Artemisia annua (C_{pA}) $= 1.8\,\frac{KJ}{Kg\,^\circ K}$

- Temprature of extractor at ambient temprature at 25°C
- The best temprature of hexane extraction process is 30°C

3.2.1. Energy Balance on Extractor I

From conservation of energy principle

$$\Delta H + \Delta KE + \Delta PE = Q - W$$

Assumption
- The system is not accelerating and failing or rising, i.e $\Delta KE + \Delta PE = 0$
- There is no moving parts at the system boundary, i.e $W = 0$

Therefore, the general energy balance reduces to: $\Delta H = Q$ and $Q = m_{cp}\Delta T$

$\dot{m}_h = V_h * \rho_h$, where $\dot{m}_h$ = mass flow rate of hexane

$$\dot{m}_h = 1133.9\frac{1}{h} * 660\frac{kg}{m^3} = 748.39\frac{kg}{h}$$

$$Q = (\dot{m}_h + \dot{m}_A)(C_{ph} + C_{pA})\Delta T$$

$$Q = \binom{187.48}{+748.39}\frac{kg}{h} * \binom{2.26}{+1.8}\frac{KJ}{Kg\,^\circ K} * \binom{30}{-25}\,^\circ K = 5.227KW$$

The amount of heat needed for heating is $18998\frac{kg}{h}$.

3.2.2. Energy Balance on Extractor II

Since the two extractors are identical their energy consumption is equal. I.e. Q= $18998\frac{kg}{h}$.

3.2.3. Energy Balance on Solvent Evaporator

Necessary data to calculate energy balance on solvent evaporator

- Volume flow rate of crude extract = $2283\frac{1}{h}$

- Density of crude extract of artemisia annua = 1250 $\frac{kg}{m^3}$

- Boiling point of hexane = 69°C
- Volume flow rate of hexane evaporated (V_{HE}) = $113.39\frac{1}{h}$

- Density of liquor which comes from solvent evaporator = $\rho_l = 1280.8\frac{kg}{m^3}$

- Volume flow rate of liquor = $2169.6\frac{1}{h}$

- Specific heat capacity of crude extract = $1.82\frac{KJ}{Kg\,^\circ K}$

- Crude extract temprature = 30°C

$\dot{m}_{HE} = V_{HE} * \rho_h$, where $\dot{m}_{HE}$ is mass flow rate of hexane evaporated

$\dot{m}_{CE} = V_{CE} * \rho_{CE}$, where the subscript CE shows

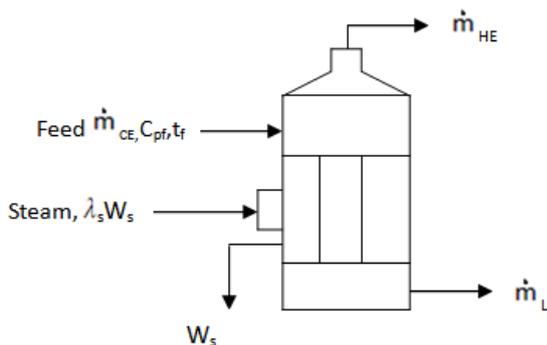$$\text{Crude Extract} = 2853.77\frac{kg}{h}$$

Figure 4. Solvent evaporator

Assumption

- There are no chemical heat effectes as the result of concentration (i.e –Ve heat of solution)
- There is no boiling point rise (BPR)
- Average specific heat = 1.0

Heat balance

$$\lambda_s W_s + \dot{m}_{CE}C_{pf}\left(t_f - t_l\right) = \dot{m}_{HE} * \lambda_l$$

Important data

Temprature, pressure (Psi), latent heat of vaporization ($\frac{Btu}{Ib}$)

$\dot{m}$ λ_l = latent heat of vaporization at 69°C= $2336.1\frac{KJ}{Kg}$

$\dot{m}$ λ_s = latent heat of vaporization at 30 = $2430.6\frac{KJ}{Kg}$

$\dot{m}_s W_s + C_{pf}(t_f - t_l) = \dot{m}_{HE} * \lambda_l$

$\dot{m}_s = 117.7\frac{kg}{h}$

The amount of steam needed for solvent evaporation is 0.0327KJ.

3.2.4. Energy Balance on Crystallizer I

Important data

- Mass flow rate of liquor = $2778.9\frac{kg}{h}$

- C_p of liquor = $1.81\frac{KJ}{Kg\,^\circ K}$

- Temprature of crystalline artemisinin = 5°C in the crystallizer
- Temprature of liquor is 30°C

$Q = m_{cp}\Delta T = 2778.9 * 1.8 * (5 - 30) = - 34.92KW$

Amount of heat loss on crystallizer to purify the liquor which comes from solvent evaporator is $125746\frac{KJ}{h}$.

3.2.5. Energy Balance on Crystallizer II

The basic information to calculate the energy balance on crystallizer II is:

- Temprature of crystalline artemisinin which comes from washer is at 25°C
- Temprature of crystalline artemisinin in crystallizer II is at 5°C
- Cp of crystalline artemisinin = $1.78\frac{KJ}{Kg\,^\circ K}$
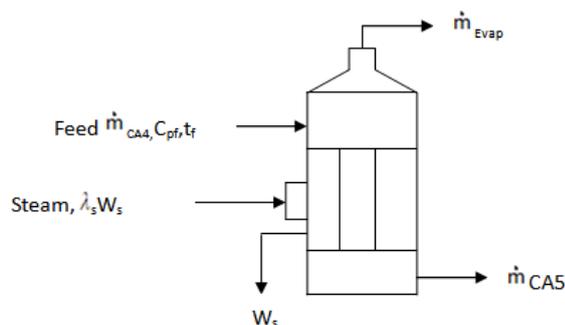
$\dot{m}_{CA} = 8.75 * 1.26 = 11\frac{KJ}{h}$

$Q = \dot{m}_{CA} C_{pCA}\Delta T = - 0.109\ KW$

Amount of heat loss on crystallizer II to purify the crude artemisinin is $392.6\frac{KJ}{h}$.

3.2.6. Energy Balance on Small Evaporator

Required data is:

- Density of crystalline artemisinin = 1250 $\frac{kg}{m^3}$.

- $V_{CA4} = 6.33 \frac{1}{h}$

- $\dot{m}_{CA4} = 7.91 \frac{kg}{h}$

- $\dot{m}_{Evap} = 0.73 \frac{kg}{h}$

- Feed temprature 25°C

Assumption

- There are no chemical heat effects as the result of concentration (i.e –Ve heat of solution)
- There is no boiling point rise (BPR)
- Average specific heat = 1.0
- Latent heat of vaporization at 66°C (λ_l)= 2344.6 KJ/Kg
- Latent heat of vaporization at 25°C (λ_s)= 2442.58 KJ/Kg

$$\dot{m}_s\lambda_s + \dot{m}_{CA}C_{pf}\left(t_f - t_l\right) = \dot{m}_{Evp} * \lambda_l$$

$$\dot{m}_s = 0.83\frac{kg}{h}$$

The amount of steam needed for small evaporator is $0.83\frac{kg}{h}$.

4. Conclusion

The extraction of artemisinin from its root source Artemisia annua is appreciable with hexane solvent with simple, emission zero and Ethiopian plants were good oil contents. By improving the de solventizer efficiency it is possible to protect the hexane emission and it can be use the spent dried leaves as an energy source. All unit operations in the extraction processes are mechanical unit operations. No reactions are taking place. Therefore, generation, consumption and accumulation terms are absent in all material balance calculations. When the numbers of crystallizers increase it was purified better and better. Energy balance calculations showed in the large plant processing the extractors consumed huge amount energy for heating when compared with other heat transfer unit operations. On the contrary, crystallizer I discharges huge energy to the environment at the purification of liquor. Significant amount of hexane was recycled from the solvent evaporator and washing tank to protect the hexane released to the environment and to save the raw hexane.

References

[1] Fabricant, D. S., and Farnsworth, N. R., The value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives. National Institute of Environmental Health Science 109(2001): 69-75.

[2] Cos, P., Vlietinck, A. J., Berghe, D. V., and Maes, L., Anti-infective potential of natural products: how to develop a stronger in vitro "proof-of-concept", Journal of Ethnopharmacology 106 (2006):290-302.

[3] Hsu E., The history of qing hao in the Chinese materia medica. Trans. Roy. Soc. Trop. Med. Hyg. 100(2006): 505-508.

[4] Gebeyaw Tiruneh, Yigzaw Kebede, Tegbar Yigzaw, "Use of Plant Artémisia annua as a natural anti-malarial Herb in Arbaminch Town (Ethiopia)", J. Health Biomed Science, 2010. Vol.2, No.2

[5] D. Sánchez, S. Quispe, P. Rosa, M. A. A. Meireles, C. Zetzl, G. Brunner; Extraction of the Antimalarial Artemisinin from Artemisia Annua L. Leaves with Supercritical CO₂: Project integrated in the German-Brazil program "SUPERNAT".

[6] Ahmed M, Determination of Artemisinin and essential oil contents of Artemisia annua L. grown in Ethiopia and In vivo Antimalarial activity of its crude extracts against Plasmodium berghei in mice, Addis Ababa University. (2008).

[7] Abebe, D, Ayehu, A, Medicinal plants and enigmatic health practice of Northern Ethiopia, Berhanena selam printing Enterprise, Addis Ababa (2013).

[8] Govindarajan, R., Vijayakumar, M., and Pushpangadan, P., Antioxidant approach to disease management and the role of "Rasayana" herbs of Ayurveda. Journal of Ethnopharmacology 99(2005): 165-178.

[9] Lombardino, J. G., and Lowe, J. A. The role of the medicinal chemist in drug discovery - then and now, Nature Reviews Drug Discovery 3(2010): 853-862.

[10] Coulson J.M., Richardson J.F., Coulson and Richardson's chemical engineering, volume 2, 3rd edition. 1995.

[11] Newman, D. J., Cragg, G. M., and Snader, K. M., The influence of natural products upon drug discovery., Natural Product Reports. The Royal Society of Chemistry 17(2009): 215-234.

[12] Subapriya, R., and Nagini, S. Medicinal properties of neem leaves: a review. Current medicinal chemistry Anticancer agents 5(2005): 149-146.

[13] Aikawa, M. The fine structure of the erythrocytic stages of three avian malarial parasites, Plasmodium fallax, P. lophurae, and P. cathemerium., The American Journal of Tropical Medicine and Hygiene 15(2006) 449-471.

[14] Dondorp, A. M., Kager, P. A., Vreeken, J., and White, N. J. Abnormal blood flow and red blood cell deformability in severe malaria., Parasitology today Personal ed 16(2009): 228-32.

[15] Geysen, H.M., Schoenen, F., Wagner, D., Wagner, R. Combinatorial compound libraries for drug discovery: an ongoing challenge. Nat. Rev. Drug Discovery 2 (3), (2002): 222-230.

[16] Romero, M.R., Effeth, T., Serrano, M.A., Castano, B., Macias, R.I., Briz, O & Martin, J.J., Effect of artemisinin/artesunate as inhibitors of hepatitis B virus production in an 'in vitro' system. Antiviral Res. 68(2005): 75-83.

[17] Tilak, K. V. B. R., N. Ranganayaki, K. K. Pal, R.De, A. K. Saxena, C. Shekhar Nautiyal, Shilipi Mittal, A. K. Tripathi & B. N. Johri. Diversity of plant growth and soil health supporting bacteria. Current Science. 89(2010): 136-150.