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KINETICS OF ULTRASOUND ASSISTED EXTRACTION OF WEDELOLACTONE FROM *Eclipta alba*

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Abstract - Ultrasound assisted extraction of wedelolactone, a major coumestan present in *Eclipta alba*, is investigated in the present work.Various process parameters such as type of solvent, power, solvent to solid ratio and extraction temperature, which affect the extraction yield, are optimized. In the ultrasound-assisted extraction with final optimized conditions, i.e., methanol as solvent, 170 W power, 60:1 solvent to solid ratio, 50 °C temperature and 60% duty cycle, amaximum extraction yieldof 0.62 mg/g is obtained in 45 minutes. The kinetic model (Peleg's model) has been used for the prediction of the yield of wedelolactone in the extract at a given time for all experimental conditions. The values of predicted yields show good agreement with the experimental data for all parameters, i.e., power, solvent to solid ratio and temperature. The extraction of wedelolactone from *Eclipta alba* is also carried out by conventional extraction methods, i.e., Soxhlet and batch extraction. Ultrasound-assisted extraction gives higher extraction yield in less time as compared to batch extraction (0.41 mg/g in 90 min) and Soxhlet extraction(0.7 mg/g in 360 min). The ultrasound-assisted extraction of wedelolactone from *Eclipta alba* is an effective way of extraction with the advantages of lower time and higher extraction.
Keywords: *Eclipta alba*; Wedelolactone; Ultrasound; Extraction kinetics; Bioseparations; Optimisation; Mass transfer.

INTRODUCTION

Eclipta alba is used in traditional herbal medicine worldwide. It is reported to possess anti-hyperglycemic, anti-hepatotoxic, antioxidant, anti-inflammatory, antifungal activities and also a good hair growth regulator and potential memory modulator (Karthikumar *et al*., 2007; Wagner *et al*., 1986; Dalal *et al*., 2010). *E. Alba* is traditionally used to calm the mind, remove memory disorders, relieve swollen glands, strengthen the spleen, stimulate digestion, as a general tonic, for treatment of edema, fevers, rheumatic joint pains, liver damage due to cirrhosis, infective hepatitis, and hepatic enlargement, enlarged spleen and skin disorders. The plant contains the alkaloid ecliptine along with other chemicals such as wedelolactone, demethylwedelolactone, wedelic acid, apigenin, luteolin, triterpenoid glucoside ecliptasaponin D, Ecliptasaponin C, daucosterol stigmasterol-3-O-glucoside etc (Dalal *et al*., 2010).

The major coumestan derivatives present in *E. alba* are wedelolactone and demethylwedelolactone. The structure of wedelolactone is shown in Figure 1. Although it possesses so many therapeutic activities, the extraction of wedelolactone has not been reported. The traditional solvent extraction technique used for extraction of natural products suffers from various disadvantages such as large solvent requirement, higher extraction time, lower yield and higher extraction temperature and thus requires development of an

*To whom correspondence should be addressed effective and economical extraction method. The novel methods that are popular for the extraction of natural products are supercritical fluid extraction (Hatami *et al*., 2012), microwave assisted extraction (Bagherian, 2011), ultrasound assisted extraction (Dey and Rathod, 2013), pressurized solvent extraction (Dunford *et al*., 2010) etc. All these techniques have their own advantages as well as disadvantages. Ultrasound assisted extraction (UAE) is one of the promising techniquesfor the extraction of natural products due to reduction in extraction time and solvent consumption. In addition, ultrasound-assisted extraction can be carried out at a lower temperature, which can avoid thermal damage to the extracts. Inspite of these advantages of UAE, extraction of wedelolactone using ultrasound has not been explored and reported in the literature. Therefore, in the present work optimization of the extraction process using ultrasound has been carried out.

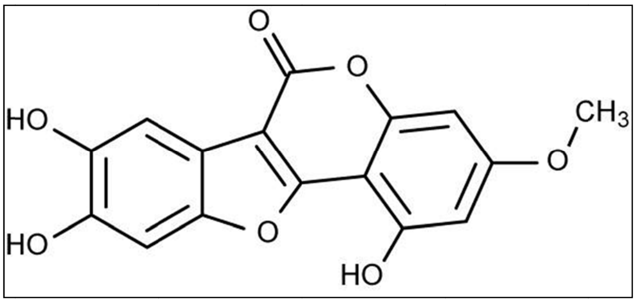

Figure 1: Structure of wedelolactone.

Ultrasound-assisted extraction of many other ingredients from natural sources has been carried out such as extraction of β-carotene from *Spirulina platensis* (Dey and Rathod, 2013), flavour compounds from spearmint (Porto and Decorti, 2009) etc. It is reported that, by the application of ultrasound, the extraction of these natural ingredients is enhanced. In ultrasound-assisted extraction, the extraction rate is enhanced due to cavitation. The cavity developed grows in size and then collapse abruptly with the release of energy, thus increasing the local temperature and pressure. Close to a solid boundary, cavity collapse is asymmetric and produces high-speed jets of liquid. The liquid jets have strong impact on the solid surface (Luque-Garcia and Luque de Castro, 2003). Therefore, greater penetration of solvent into cellular material occurs and this improves the release of cell content into the bulk medium. Similarly, the physical effects such as liquid circulation and turbulence produced by cavitation help in increasing the contact surface area between the solvent and targeted compounds by permitting greater penetration of solvent into the sample matrix.

Kinetic studies play a very important role in extraction processes and the knowledge of extraction rate, i.e., whether the extraction process is slow or fast can be easily obtained from the kinetic study of the process. Kinetic studies help in understanding various factors that affect the extraction rate. Thus, it is important to study the kinetics of the extraction process. Although various research papers are available which report the ultrasound-assisted extraction, very few papers report the kinetic aspects. To the best of our knowledge, the ultrasound-assisted extraction kinetics of wedelolactone have not been reported up to now. Thus, the objective of the present study is to develop the optimized ultrasound-assisted extraction process for the extraction of wedelolactone from *E. alba* leaf powder and to study the kinetics of this process.

MATERIALS AND METHODS

Material

Eclipta alba leaf powder was purchased from a local market (size: 40-50 mesh). Methanol, ethanol and acetone used as solvent for extraction were of analytical grade.Water used as a solvent was freshly prepared de-ionized water from Millipore Milli – Q 50. Methanol and acetic acid used as solvent for High Pressure Liquid Chromatography were analytical grade purchased from Hi Media Ltd., Mumbai, India. Standard wedelolactone (≥ 99%) was purchased from Natural Remedies Pvt. Ltd., Bangalore, India.

Apparatus

Ultrasound-assisted extraction was carried out in a dual-frequency ultrasound cleaning bath (Model 6.5l200 H, Dakshin, India) of internal dimensions 230 mm x 150 mm x 150 mm and tank capacity of approx. 6.5 litres, with a rated ultrasonic power of 200 watts and frequencies of 25 kHz and 40 kHz, equipped with heater and digital temperature controller/indicator. A selector switch is provided on the panel to select one operating frequency at a time. Power variation is possible by varying the input AC voltage through an auto-transformer.

Ultrasound-Assisted Extraction

The extraction was carried out in a glass vessel (flat bottom, 3.5 cm internal diameter and 16 cm height) kept in an ultrasound bath. The extraction vessel was kept at the position and depth (2.54 cm above the bottom of the bath)optimized previously earlier (Kulkarni and Rathod, 2014).1 g of the *E. alba* leaf powder was taken in a glass vessel and 40 mL solvent was added to it. In order to avoid the loss of solvent, the glass vessel was covered with a lid before it was placed in an ultrasound bath. The ultrasound was introduced for 80 minutes. Very small aliquots were withdrawn at a specific time interval, so as not to disturb the volume. These samples were then filtered, diluted and analysed using HPLC. Extraction experiments were carried out to study the effect of different parameters that affect the extraction yield, such as different solvent, extraction time and physical parameters like power, solvent to solid ratio and extraction temperature. Input Power was varied from 100W to 170 W. Solvent to solid ratio was varied from 20:1 to 80:1, keeping all other parameters constant. The bath temperature was varied from 30 °C to 70 °C using the heater provided with the ultrasound cleaning bath and all other parameters were kept constant.

Soxhlet Extraction

A conventional Soxhlet apparatus, which consisted of a distillation flask placed in an oil bath, thimble holder and the condenser, was used for extraction of wedelolactone from *E. alba*. 2 g of the powder of *E. Alba* leaves was packed in filter paper and placed in a thimble holder and 200 mL solvent was placed in the distillation flask. When the boiling of the solvent in the distillation flask started, the thimble holder was filled with condensed fresh solvent. Very small aliquots were withdrawn after each extraction cycle and analysed using HPLC. The experiment was continued until complete exhaustion of the raw material.

Batch Extraction

Batch extraction was carried out in a glass reactor of capacity 150 ml equipped with a six bladed (pitched blade) glass turbine for agitation. 1 g of the *E. Alba* leaf powder was placed in a glass reactor and 40 mL solvent added to it. The mixture was agitated for 3 hrs. Very small aliquots were withdrawn after a specific interval of time and filtered. The clear liquid samples are then diluted and analyzed for wedelolactone using HPLC. Different parameters affecting the extraction such as extraction time, solvent to solid ratio, speed of agitation and extraction temperature were optimized and the final extraction experiment at the optimized conditions was carried out to compare it with ultrasound assisted extraction.

Analytical Method

The wedelolactone was analyzed using HPLC. The HPLC instrument was anAgilent 1260 series HPLC system equipped with a diode-array UV–vis detector (DAD, model G1315D). Analysis was performed on a XBD-C18 reversed-phase column (Eclipse, 250×4.6 mm, 5 μm) at the wavelength of 351 nm. The mobile phase consists of a mixture of 60% methanol and 40% acidified water (1% acetic acid) and the flow rate was maintained at 1 ml/min.

Kinetic Model

The well-known semi-empirical kinetic model introduced by Peleg (1988) is widely used to explain the extraction curves of biological materials from plant sources because of the similarity to the shape of sorption curves (Peleg, 1988). The model describes the sorption isotherms of materials as follows:

$$C_t = C_0 + \frac{t}{K_1 + K_2 t} \tag{1}$$

where C_t is the concentration of wedelolactoneat time t (mg/g), K_1 is Peleg's rate constant (min g/mg) and K_2 is Peleg's capacity constant (g/mg).

The C_0 term can be omitted from Peleg's equation as the initial concentration of the target solute is zero in the extraction solvent because fresh solvent is used at the beginning. The extraction occurs in two stages, i.e. at the very beginning it is first order and in the latter phase of the process decreases to zero order. The modified Peleg's equation representing the concentration of the target solute in the extraction solvent against time is shown below:

$$C_t = \frac{t}{K_1 + K_2 t} \tag{2}$$

C_t can be calculated using the above mentioned Eq. (2) by determining the K_1 (Peleg's rate constant) and K_2 (Peleg's capacity constant) values by plotting the graph of $1/C_t$ vs. $1/t$.

The Peleg capacity constant K_2 relates to the maximum of the extraction yield, i.e., the equilibrium concentration of extracted wedelolactone (C_e). When t tends to ∞, Eq. (2) gives the relations between equilibrium concentration and K_2 as:

$$C_{t \to \infty} = C_e = \frac{1}{K_2} \quad (3)$$

RESULTS AND DISCUSSION

Soxhlet and Batch Extraction

Soxhlet extraction for 6 hours resulted in awedelolactone yield of 0.70 mg/g. This is considered as the maximum obtainable yield, i.e., 100% extraction and used for comparison with the yield obtained by UAE. In batch extraction, process parameters which affect the extraction yield, i.e., extraction time, speed of rotation (200, 400 and 600 rpm), solvent to solid ratio (20:1, 40:1, 60:1 80:1, and 100:1) and temperature (30, 40, 50,60 and 70 °C) were varied and optimized. Final optimized conditions are found as: extraction time 90 min, speed of agitation 400 pm, solvent-to-solid ratio 80:1 and temperature 70 °C. The final experiment using these conditions resulted in the yield of 0.41 mg/g as shown in Figure 2.

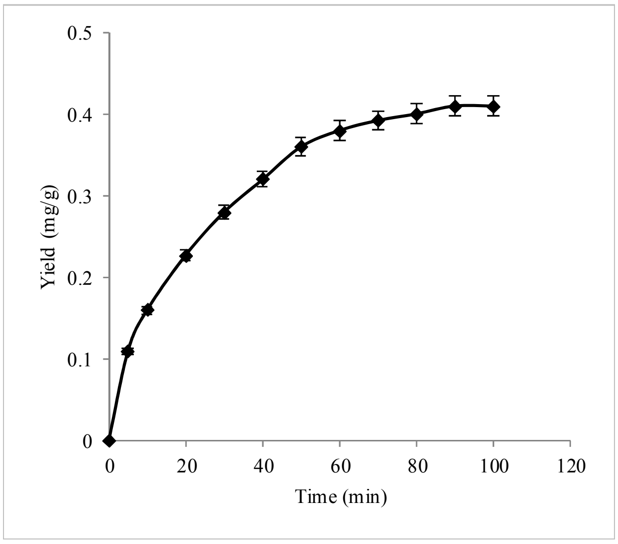

Figure 2: Batch extraction of wedelolactone at the optimized conditions.

Effect of extraction time in UAE

To identify the equilibrium time of the extraction process, the effect of time on extraction yield was studied. Other parameters were: power 170 W, solvent to solid ratio 40:1, temperature 30 °C. The variation in the amount of wedelolactone extracted per g of *E. alba* with time is shown in Figure 3. It can be seen that the yield of wedelolactone increases rapidly upto 45 min and thereafter a gradual increase in the yield is observed. This trend matches with the classical extraction process from the plant material.

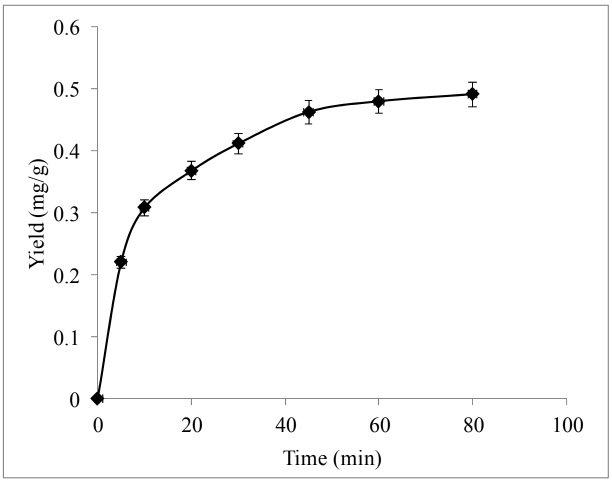

Figure 3: Effect of time on the extraction yield of wedelolactone.

Effect of Different Solvents in UAE

The solubility of a certain compound in a particular solvent varies with the nature of the solvent, i.e., a polar solute is soluble in polar solvents whereas a non-polar solute dissolves in non-polar solvents. But the complex structure of natural products and chemical characteristics of the solvent make it difficult to predict the chemical interaction between them. The effect of different solvents on the extraction yield was investigated and the results are shown in Figure 4. Different solvents like water, methanol, ethanol, and acetone were used for the extraction of wedelolactone. All the experiments were carried out for 45 minutes at a power of 170 W, solvent-to-solid ratio of 40:1 and 30 °C. Figure 3 shows that the maximum extraction yield is obtained using methanol followed by ethanol. But the extraction of wedelolactone is very low with water and acetone. Solubility of the solute in the solvent plays an important role in the extraction process. The solubility of wedelolactone is higher in methanol than in other solvents. Polarity of the solvent is also a significant factor which affects the extraction yield. There are several parameters such as solvent viscosity, surface tension and vapour pressure which affect the cavitation and hence extraction yield. It has been reported by Luo *et al*. (2013) that the solvent viscosity, surface tension and vapour pressure affect the cavitation. A solvent that has high surface tension and high viscosity probably has an increased energy barrier for the formation of cavities, which is unfavorable for cavitation (Luo *et al*., 2013). The higher extraction obtained for methanol is probably due to its higher polarity, lower viscosity (0.544 mPa s) and lower surface tension (22.07 mN m^{-1}) as compared to other solvents. Hence for further experiments, methanol was used as solvent.

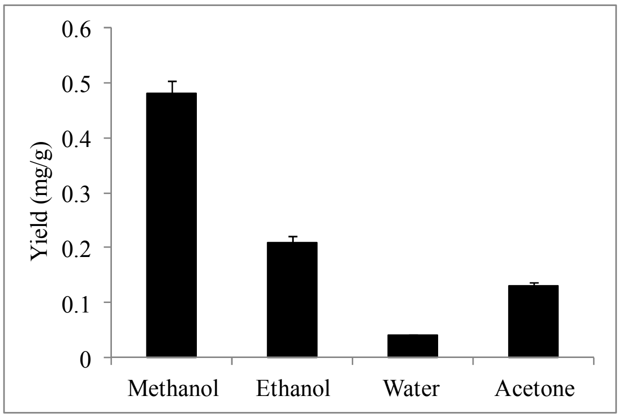

Figure 4: Effect of different solvents on the extraction yield of wedelolactone.

Optimization of Operating Parameters of UAE and Validation of the Kinetic Model

The operating parameters which affect the extraction yield are power, solvent to solid ratio and temperature. Hence these parameters are varied and optimized in UAE. The experimental data obtained are used to predict the parameters of Peleg's kinetic model, i.e., K_1 (Peleg's rate constant) and K_2 (Peleg's capacity constant) from the slope and the intercept of the graphs of $1/Ct$ vs. $1/t$ at different powers, solvent to solid ratios and temperatures. Values of K_1 and K_2 are reported in Table 1. Then these values are used for the prediction of extraction yield of wedelolactone for different operating parameters.

Effect of Power

The effect of ultrasound power on the extraction of wedelolactonewas studied by varying input power at 100, 140 and 170 W. Other experimental parameters were a frequency of 25 kHz, temperature 30 °C and solvent to solid ratio 40:1. The actual power dissipated at these input powers was estimated calorimetrically and the values were 31.7, 37.3 and 46.5 W for input powers of 100, 140 and 170 W, respectively. The results are shown in Figure 5. When the ultrasound power was increased from 100 to 170 W with an extraction time of 45 min, the yield of wedelolactone increased. The vibration caused by ultrasound waves that occurred at the interfaces between solvent and solid matrix affects the extraction. For a given medium and a fixed radiation area, the vibration is proportional to the ultrasound power. At higher power, larger amplitude ultrasound waves are generated through the solvent, which results in formation of a greater number of bubbles and collapse. The generated shock wave causes penetration of solvent into cells, which accelerates mass transfer rate and the rate of extraction. Hence the maximum yield is obtained at the highest power input.

By applying Peleg's kinetic model described above for this experimental data, values of K_1 and K_2 were calculated. These values were then used to predict the yield of wedelolactone at different time and at equilibrium. Values of K_1, K_2 and C_e are tabulated in Table 1. In Figure 5, experimental and predicted yields of wedelolactoneare plotted where bullets represent the experimental data and the solid line represents the predicted yield. It can be seen from the Figure that there is a good agreement between experimental and predicted yield of wedelolactone.

Table 1: Effect of ultrasonic power on the kinetic parameters and calculated C_e (mg/g).

Power	K_1	K_2	Extraction extent C_e
100	18.615	2.198	0.4548
140	16.444	2.079	0.4808
172	13.259	1.939	0.5154

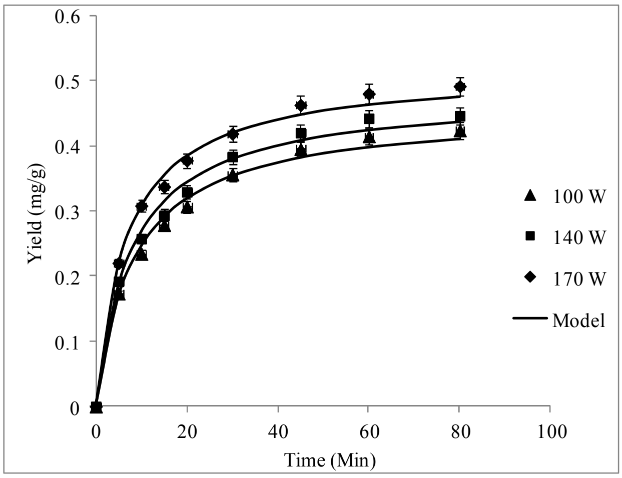

Figure 5: Effect of power on the extraction yield of wedelolactone.

Effect of Solvent to Solid Ratio

The amount of solvent available for the solute to leach out is an important factor affecting the extraction. Therefore, the effect of solvent to solid ratio on extraction yield was investigated and the results are shown in Figure 6. It is observed that the amount of wedelolactone extracted per gram of *E. alba* increases when the solvent to solid ratio is increased from 20:1 to 60:1; for 80:1 ratio no significant increase in the extraction yield is observed. When the solvent to solid ratio increases it provides a larger concentration gradient between solute and solvent, hence the mass transfer increases. Once there is a sufficient amount of solvent available for extraction, further addition of solvent does not affect the extraction yield. As there is a marginal change in extraction yield for 60:1 and 80:1ratio, the former was taken as the optimized ratio and used for all further experiments.

The values of K_1, K_2 and C_e were calculated from the model and listed in Table 2. To validate the kinetic model, values of the concentration of wedelolactone in the extract at different times for different solvent to solid ratios are calculated from the model and plotted in the graph. Figure 6 shows that there is a good agreement between the experimental and predicted values of concentration of wedelolactone in the extract.

Table 2: Effect of Solvent to solid ratio on the kinetic parameters and calculated C_e (mg/g).

Solvent to solid ratio	K_1	K_2	Extraction extent C_e
20	18.119	2.552	0.3918
40	13.259	1.94	0.5154
60	8.853	1.863	0.5367
80	7.572	1.814	0.5512

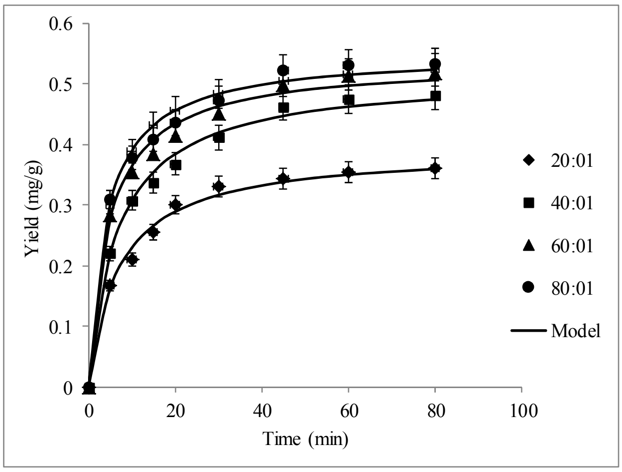

Figure 6: Effect of solvent to solid ratio on the extraction yield of wedelolactone.

Effect of Temperature

It is well known that the solubility of solute increases with temperature and thus the extraction yield also increases. The temperature also influences the vapour pressure and physical properties like viscosity, surface tension of the solvent and the cavitation in the case of ultrasound-assisted extraction. Therefore, the effect of temperature on the extraction yield of wedelolactone was studied and the results are shown in Figure 7. Extraction was carried out at different bath temperatures, i.e., 30 °C, 50 °C and 70 °C. It is observed that the extraction yield increased with an increase in temperature which is a result of increased solubility at higher temperature. However, the change in extraction yield is marginal from 50 to 70 °C. Vapor pressure significantly influences the occurrence and the intensity of acoustic cavitation. When the temperature is low, bubbles are few but they collapse with relatively high intensity, which enhances the cell disruption and thus the mass transfer. But, when temperature is high, though more bubbles are created, they collapse with less intensity. Hence, at higher temperature the change in extraction yield is marginal and thus the 50 °C temperature is taken as optimum.

The values of K_1 and K_2 are calculated from the model and listed in Table 3. Values of the concentration of wedelolactone in the extract at different time intervals for different temperatures are calculated from the model and plotted in Figure 7. This shows that agreement between the experimental and predicted values of the concentration of wedelolactone in the extract is quite good.

Table 3: Effect of temperature on the kinetic parameters and calculated C_e (mg/g).

Temperature (°C)	K_1	K_2	Extraction extent C_e
30	13.884	1.967	0.5083
50	11.711	1.619	0.6176
70	9.917	1.538	0.6501

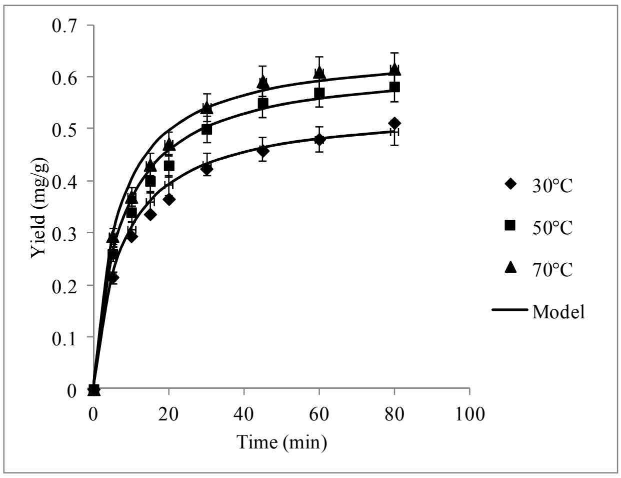

Figure 7: Effect of temperature on the extraction yield of wedelolactone.

The Effect of Temperature on the Peleg's Rate Constant

To get a clear idea of the energy involved in the extraction process, the comparison of the activation energies for ultrasound and conventional extraction was studied. The influence of temperature on the initial extraction rate ($1/K_1$) (1/Peleg's rate constant) is assessed by the linearized Arrhenius equation, giving the relationship between $1/K_1$ and extraction temperatures as follows:

$$\ln\left(\frac{1}{K_1}\right) = \ln(A) - \frac{E_a}{RT} \tag{4}$$

where K_1 is the Peleg rate constant (min g/mg UA), A is a constant called as frequency factor (min^{-1}), Ea the activation energy (J/mol), R the universal gas constant (8.314 J/(mol K)), and T the absolute temperature (K).

The plot of the $\ln(1/K_1)$ vs. $1/T$ should result in a straight line with the negative of the slope equal to Ea/R and intercept equal to $\ln(A)$. The activation energies of the ultrasound-assisted extraction and batch extraction were found to be 7.26 kJ/mol and 10.95 kJ/mol, respectively. From these results, we can conclude that the initial extraction rate is more temperature sensitive for the conventional batch extraction process than for UAE. This means that an increase in the temperature will affect the initial extraction rate of the batch extraction process more than in the case of UAE. Similar results are also reported by Vetal *et al*. (2013) for the extraction of ursolic acid from *Ocimum sanctum* by ultrasound.

Effect of Duty Cycle

The duty cycle, i.e., ON–OFF timing, of the ultrasonicator must be optimized for better extraction, as an unnecessary increase in the ON timing would lead to excessive heating and excessive energy consumption. The effect of duty cycle on the extraction yield of wedelolactone was studied at 20%, 40%, 60%, 80% and 100% duty cycles and results are presented in Figure 8. It can be seen from the figure that the extraction yield increased with an increase in the duty cycle from 20% to 60% and then became almost steady up to 100% duty cycle. Hence the 60% duty cycle was selected as the optimum value as there is no change in extraction yield after 60% duty cycle. Similar results are also reported by Dey and Rathod (2013), who carried out the extraction of β-carotene from S*pirulina platensis* and Sun *et al*. (2011) who investigated the effects of different factors of ultrasound treatment on the extraction yield of all-trans-β-carotene.

Comparison of UAE with Traditional Extraction Techniques

In order to compare the efficiency of UAE, the extraction of wedelolactone was also performed using conventional techniques, viz., Soxhlet and batch extraction. Table 4 shows the optimum results obtained with the Soxhlet extraction, batch extraction and UAE. It is observed that the time taken by UAE is much less compared to Soxhlet extraction, i.e., using UAE, a yield of 0.62 mg/g is obtained within 45 min of extraction time whereas the Soxhlet extraction required 6 hrs for extraction of 0.70 mg/g of wedelolactone. In case of batch extraction the time required for extraction is double that for UAE and the extraction yield is stilllower than that in UAE. The solvent consumption was also reduced with the use of ultrasound. In batch extraction 80 mL of solvent is required per g of the raw material; however, in UAE the solvent requirement was reduced to 60 mL per g of raw material. Thus, UAE is more efficient extraction technique than conventional techniques in terms of time and solvent required and yield.

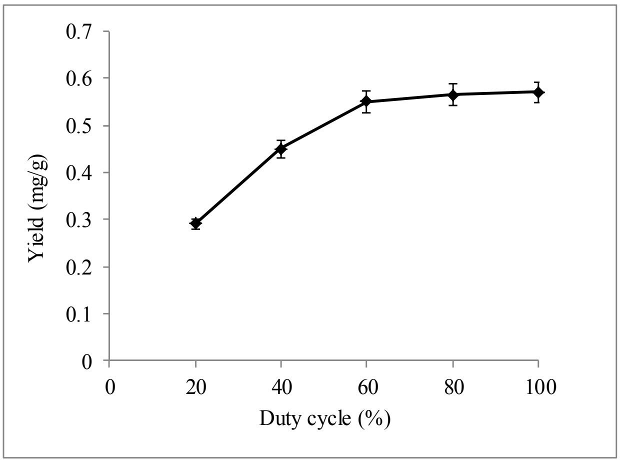

Figure 8: Effect of duty cycle on the extraction yield of wedelolactone.

Table 4: Comparison of UAE, batch extraction and Soxhlet extraction.

Technique of extraction	Time required (min)	Solvent required (ml/g)	Temperature (°C)	Yield (mg/g)
Soxhlet	360	-	90	0.70
Batch	90	80	70	0.41
UAE	45	60	50	0.62

CONCLUSIONS

In the present work, extraction of wedelolactone from *Eclipta alba* leaves powder was successfully carried out using ultrasound. In order to obtain maximum yield, the impact of various parameters such as type of solvent, input power, temperature and solvent to solid ratio was investigated. As the power input increased, the extraction yield increased and the highest extraction yield was obtained at 170 W. Extraction initially increased rapidly with an increase in solvent-to-solid ratio and then gradually, giving maximum yield at a 60:1 ratio. It was also observed that the extraction yield increases with temperature and the initial extraction rate of the conventional process was more temperature dependent than UAE. With optimized conditions, i.e., methanol as solvent, 170 W power, 60:1 solvent to solid ratio, 50 °C temperature, a 0.62 mg/g yield was obtained. The extraction yield obtained with ultrasound-assisted extraction was compared with that of Soxhlet extraction and batch extraction. Ultrasound-assisted extraction gavea higher extraction yield with lower solvent consumption as compared to batch extraction. It also required less time as compared to both Soxhlet and batch extraction. Hence, it can be concluded that the ultrasound-assisted extraction of wedelolactone from *Eclipta alba* is an effective method for extraction with the advantages of lower time and solvent requirement and higher extraction yield. Peleg's kinetic model was used for the prediction of yield of wedelolactonein the extract at a given time for all experimental conditions.The values of predicted yields showed good agreement with the experimental data for all parameters, i.e., power, solvent to solid ratio and temperature.

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