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Research paper

Optimization of Supercritical Extraction Conditions of *Senna Alata* and Evaluation of Biological Activity

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Abstract

Supercritical fluid extraction (SFE) offer faster extraction process, decreased solvent usage and more selectivity on desired compounds. In this present study, the influence of pressure (100, 200 and 300 bar) and temperature (40, 50 and 60˚C) on the *Senna alata* crude yield were investigated with fixed supercritical carbon dioxide (SC-CO_2) at the flow rate of 35 g/min. The parameters were optimised and modelled using response surface methodology (RSM) and central composite design (CCD). The analysis of variance (ANOVA) experimental design consists of 13 experimental runs with 5 replicates at the central points. Well-fitting quadratic model were successfully established for crude extract through backward elimination. The optimum crude extract yield pointed out by RSM was at the pressure of 300 bar and temperature 40˚C respectively. Extraction yields based on SC-CO_2 varied in the range of 0.28 to 3.62%. The highest hyaluronidase inhibition activity and total flavonoids content obtained by *S.alata* crude extracts were 41.19% and 52.53% w/w, respectively. SC-CO_2 proves to have great potential for extraction of yield, hyaluronidase inhibition activity and total flavonoids content for *S.alata*.

Keywords: *Supercritical carbon dioxide conditions; hyaluronidase activity; total flavonoids content.*

1. Introduction

Extraction method and extracting solvent are important for quantity and quality of extracts. Hence, appropriate extraction method for each plant should be applied to obtain the highest amount of bioactive compounds. Sequential extractions using solvents such as petroleum ether, chloroform, methanol and ethanol have been found to be effective against pathogenic bacteria (Chatterjee et al., 2012; Gritsanapan & Mangmeesri, 2009; Ehiowemwenguan et al., 2014; Hong & Lyu, 2011) as well as against a few fungi that could cause dermatophytic diseases such as *C. albicus, T. mentagrophyte, A. niger, D. congolensis, C. albicans* etc (Alalor et al., 2012; Ali-Emmanuel et al., 2003; Owoyale et al., 2005). However, recent studies have shown that supercritical fluid extraction (SFE) offers vast difference over solvent-based extraction techniques. Compared to the conventional solvent method, extraction via supercritical fluid provides the following advantages: faster extraction process, more selectivity of desired compounds, decreased solvent usage and lower costs for solvent disposal (Wright & DePhillipo, 2015; David & Selber, 1996). In addition, SFE requires very little to no dry-down time prior to the analysis and hence limits the thermal degradation (Capuzzo et al., 2013). There are many literature about the natural materials extraction with SFE such as *Marchantia convoluta* (Chinese herb) (Xiao et al., 2007).

SFE of plant material is a growing topic of interest with solvents such as carbon dioxide (CO_2), propane, butane or ethylene. It allows separation by using the supercritical fluid as the solvent. A substance is considered to be in supercritical condition when it is above its critical temperature and critical pressure. The main and commonly used solvent is CO_2. It is a cheap, eco-friendly, and generally recognised as a safe component. SFE using CO_2 is also attractive because of its high diffusivity and allows the extraction of easily oxidised compounds in natural products (David & Selber, 1996; Xiao et al., 2007; Wright & DePhillipo, 2015). However, conventional supercritical fluid carbon dioxide (SC-CO_2) suffers from low polarity which affects the efficiency of extracting the compound of interest.

The genus *Senna* (Fabaceae) is represented in Southern North America, Brazil by its beautiful yellow flowering shrub that grows about 1 to 2 m in height. It produces a wide range of bioactive molecules that can be found mainly in its leaves rather than its flowery shrub, making it a rich source for various types of anti-inflammatory and antibacterial traditional medicines. Thus *S.alata* plays an important role in drug development in pharmaceutical industries. It has been cultivated to treat skin diseases, ringworms, fever, constipation etc. (Mohideen et al., 2005). The main activity of its leaves is associated with the presence of numerous active chemical components such as phenols, tannins, saponins, alkaloids, steroids, flavonoids and carbohydrates. The major technical challenge in the application of extraction process using supercritical fluid is optimising variable combinations of temperature and pressure to increase in the solvent's effectiveness (David and Selber, 1996).

Hence, this paper is to investigate and optimise important variables such as pressure and temperature of the supercritical fluid carbon dioxide extraction of *S.alata* as well as to discover the relationship of the variables with hyaluronidase inhibitory activity and total flavonoid contents.

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2. Methodology

2.1. Chemicals and Materials

Dried samples of *Senna alata* was purchased from HERBagus Sdn. Bhd., Penang. Hyaluronidase (bovine testes, type 1-S), hyaluronic acid (rooster comb), bovine serum albumin (BSA) and ammonium acetate were purchased from Sigma Chemical Co. Apigenin was isolated from parsley through acid hydrolysis of apiin.

2.2. Extraction Preparation

S.alata extraction was carried out in a batch system using Supercritical Fluid Extraction (SFE) system (SFE 500MR, Thar Technology) including 500 mL stainless steel extraction vessel, automated back pressure regulator (ABPR) and, a high pressure pump. Figure 3.1 shows the scheme of supercritical CO_2 apparatus. A supercritical non-polar extracting solvent such as carbon dioxide (CO_2) was used for extraction system. 130 g of sample was charged to the extraction vessel. After recirculating chiller to 3°C, the CO_2 gas was liquefied and continuously supplied into the extraction vessel using a high-pressure pump. Experimental extraction condition were optimised according to Xiao et al. (2007) with minor modifications between two parameters; pressure and temperature. Supercritical fluid extractions were conducted at pressures of 100, 200 and 300 bar while 40, 50 and 60°C for temperatures, respectively. The supercritical CO_2 was maintained at 35 g/min. The *S.alata* samples were soaked in the solvent for 30 min (static extraction) to equilibrate the mixture at desired temperature and pressure (Bimakr et al., 2013). The static extraction time was applied for each run at respected temperature and pressure. Released solutes containing CO_2 extracts was collected after the dynamic extraction time (1 hour) and into a pre-weighed flask. The fixed dynamic extraction time was applied for each run.

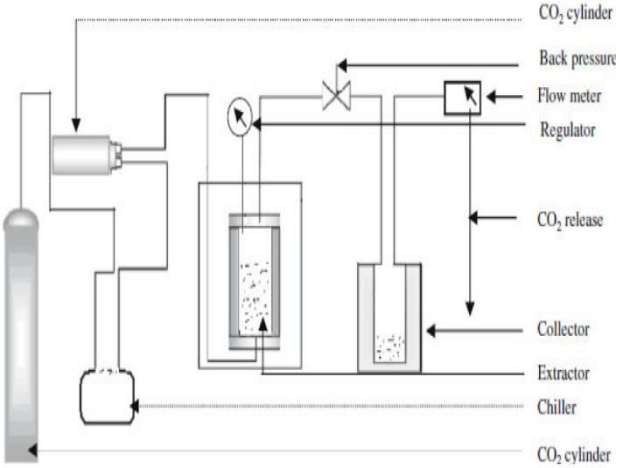


Fig. 1: Supercritical CO_2 apparatus (Pradhan et al., 2010).

2.3. Measurement of Crude Extraction Yield (CEY)

The extracts were weighed gravimetrically and then the CEY was calculated according to the following equation:

$$CEY = \frac{m_e}{m_s} \times 100\% \quad (1)$$

where m_e is the crude extract mass (g) and m_s is the dried sample mass (g). The measurement was performed in triplicate and the mean values of CEY were expressed in percent (g-extract/g-dried sample).

2.4. Optimization Analysis with RSM

The SC-CO_2 extraction parameters were optimised by applying response surface methodology (RSM). The parameters which included include pressure (100 - 300 bar) and temperature (40°C - 60°C) were varied to achieve the highest amount of crude oil from *S.alata* sample. A central composite design (CCD) with 4 axial points and 5 central points were used for designing the experimental data. The mathematical model for each run were predicted using multiple regression model and the following second-order polynomial model was fitted to the data:

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum_{i=1}^{j-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \quad (2)$$

where Y is predicted response, β_0 is offset term, β_j is the regression coefficients for linear effect term, β_{jj} is quadratic effects and β_{ij} is interaction effects. In this model, Xj and Xi are the independent variables. The experimental data for each run was analysed for the F-test of significance and was refitted only to significance higher than or equal to 5% ($p \leq 0.05$) (Paulucci et al., 2013). The model adequacy was then determined using the coefficient of determination (R^2). Lastly, an optimisation was carried out to interpret the optimal level of independent variables achieving the maximum desired response goal.

2.5. Hyaluronidase Assay

The assay was performed according to Sigma-Aldrich protocol with slight modifications (Ling et al., 2003). The assay medium containing hyaluronidase in cold 20mM sodium phosphate buffer (pH 7 at 37°C) with 77 mM sodium chloride and 0.01% (w/v) BSA was pre-incubated with 25 µL of sample compound (in DMSO) for 10 min at 37°C.Then the incubated assay was mixed with 0.03% (w/v) hyaluronic acid solution in 300 mM sodium phosphate (pH 5.35 at 37°C) and incubated further for 45 min at 37°C. The reaction mixture was precipitated with 1 mL of 0.1% BSA in 24 mM sodium acetate and 79 mM acetic acid (pH 3.75) (Acid albumin solution). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The reference value for maximum inhibition was held for absorbance in the absence of enzyme. The inhibitory activity of sample compound was calculated as the percentage ratio of the absorbance in the presence of sample compound versus in the absence of enzyme. The enzyme activity was checked by pre-incubating the enzyme in DMSO and following the procedures above. The percentage ratio of the absorbance of the presence of enzyme versus absence of enzyme should be 15-20%. The performance of the assay was verified using apigenin as a reference following the same procedures. The results were expressed as the mean of the Mean ± SEM. of three independent experiments measured in triplicates.

2.6. Total Flavonoids Content

Flavonoids content was determined by aluminium chloride colorimetric method (Chang et al., 2002) with a bit of modification. 0.5 mL of sample extract was mixed with 1.5 mL methanol, 0.1 mL 10% aluminium chloride, 0.1 mL 1M potassium acetate and 28 mL distilled water. After standing at room temperature for 30 min, the absorbance was measured at 415 nm. The calibration curve was prepared by using rutin at concentrations of 10 to 50 ppm in methanol. Total flavonoids content were expressed as a percentage of the weight of rutin equivalent to the dry weight of the sample (% w/w).

3. Results and Discussion

3.1. Response Surface Methodology

For this study, central composite design (CCD) was applied to model the CEY by using SC-CO_2 extraction. 13 experiments were assigned which included 4 axial points, 4 factorial points and 5 central points based on the CCD. Two factors (pressure and temperature) were set and three level code values (-1, 0, +1) CCD was assigned to determine the most practical and desirable combination effect of both extraction parameters. The layout of the CCD and the results obtained with each run are illustrated in Table 1. The results showed that the yield of *S.alata* crude extract, generated from different combinations of extraction conditions via SC-CO_2 was found

Table 1: Effect of extraction pressure and temperature on the crude extraction yield of *S.alata* by supercritical fluid extraction with carbon dioxide.

Run	Coded parameter		Actual parameter values		Crude extraction yield (%)
	A	B	A	B	
1	-1	-1	100.00	40.00	1.19
2	0	-1	200.00	40.00	2.31
3	-1	+1	100.00	60.00	0.94
4	0	0	200.00	50.00	1.21
5	+1	0	300.00	50.00	3.61
6	+1	+1	300.00	60.00	3.62
7	0	0	200.00	50.00	1.23
8	0	0	200.00	50.00	1.26
9	0	+1	200.00	60.00	0.92
10	-1	0	100.00	50.00	1.09
11	+1	-1	300.00	40.00	3.59
12	0	0	200.00	50.00	1.25
13	0	0	200.00	50.00	1.27

to be from 0.28 to 3.62 (%). By using multiple regression analysis, the best fitting models were determined with backward elimination. Analysis of variance (ANOVA) was used to estimate the significant relationships of the main effects and interactions. ANOVA for response surface quadratic models determined that the models were significant with $P<0.05$. The validity of data depended on the high coefficient of determination R_2 and the adjusted R_2. Adjusted R_2 is a measure of the amount of variation about the mean explained by the quadratic model (Kar et al., 2009). The accuracy of the empirical model to actual data can be indicated with value R_2 being closer to unity (Zhang et al., 2007). In other words, the regression model can be considered as giving a good response to the statistical data if the value for the response variable was higher than 0.75%. In this case, the R_2 value for CEY was 0.9534 while adjusted R_2 was 0.9202, which indicates that the regression model is a good fit. This also means that the R_2 specifies 95.34% of CEY is correlated with the independent variables and only 4.66% of the total variations could not be explained by the model. The simultaneous rise of both R_2 and adjusted R_2 in the data indicates the accuracy and is a good estimation of it. ANOVA (Table 2) also showed that quadratic regression models for crude recovery were significant with $P>0.0002$.

Table 2: ANOVA for response surface quadratic model for crude extraction yield by supercritical fluid extraction with carbon dioxide.

Source	Sum of squares	Degree of freedom	Mean square	F value	Prob>F
Model	13.34	5	2.67	28.67	0.0002
Residual	0.65	7	0.093		
Lack of Fit	0.65	3	0.22	374	<0.0001
Pure error	0.0023	4	0.00058		
Total	13.99	12			

3.2. Effects of Pressure and Temperature on SC-CO_2 Crude Extraction Yield

The influences of pressure (A) and temperature (B) were investigated. The functional correlation between temperature and pressure on the SC-CO_2 CEY from *S.alata* was determined at temperatures 40˚C to 60˚C and at pressures 100 and 300 bar, respectively. The results are obtained and collected in Table 1 where the crude recovery is described as a percentage of oil per gram dried material. The highest CEY for this experiment was 3.62% obtained at 300 bar and 60°C, followed by 300 bar and 50°C while the lowest CEY was obtained at 200 bar at 60°C. From the results, it can be indicated that the pressure and temperature have significance on the crude yield ($P<0.05$). The CEY increased gradually from low to high level (-1 to +1) of pressure (A: 100 to 300 bar). As shown in Figure 2a, pressure have a significant positive effect on the CEY as it increases. This is most likely due to increased solvent power and the solubility of *S.alata* crude to the supercritical fluid. Hence improving in the percentage of CEY during extraction (Siti Hafsah et al., 2016). Gopalan et al. (2000) also claimed that the solubility of the oil/crude could change due to increasing pressure during extraction. Murthy

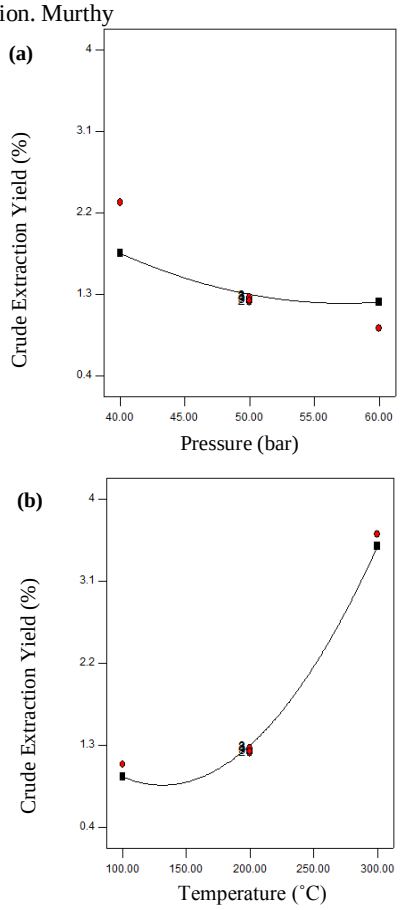


Fig. 2: (a) Effect plot of pressure (A) on crude extraction yield. (b) Effect plot of temperature (B) on crude extraction yield.

and Manohar (2014) studied the supercritical carbon dioxide extraction from Mango Ginger Rhizome to optimise the amount of total extraction yield and total phenolic content by RSM. They found that the extraction yield increased with higher temperature and pressure simultaneously due to increasing in the vapour pressure of active components in the extract. Figure 2b shows that the CEY decreased gradually from low to high level (-1 to +1) of temperature (B: 40°C to 60°C). The yield decreased may be due to reduced density of carbon dioxide as the temperature rises (Abdalbasit et al., 2010). The pressure (A) and quadratic terms for pressure (A_2) has significant positive effect on CEY due to the P values being well below the 0.05 significance level (Table 3), while the temperature (B) and quadratic terms for temperature (B_2)

have a negative effect on the CEY. Although temperature (B), combination terms of pressure and temperature (AB) and quadratic terms for temperature (B_2) have an insignificant (P>0.05) effect on CEY, they were not removed by backward elimination to support the hierarchy of the model.

To evaluate the interaction between pressure and temperature on the CEY, response surface plot was constructed at a constant carbon dioxide flow rate at 35 g/min (Figure 3). The predictive model was constructed using the actual levels of the studied factors. Based on the response surface plot, it was shown that CEY increased at high level (+1) of pressure (300 bar) and low level (-1) of temperature (40°C). The CEY is decreased when pressure is below high level (+1) and temperature is beyond the low level (-1). Response surface was constructed based on the second order polynomial equation by backward elimination:

$$Y = +9.10 - 0.03A - 0.23B + 7E^{-5}AB + 9.20E^{-5}A^2 + 1.85E^{-3}B^2 \quad (3)$$

3.3 Optimisation of Crude Extraction Yield

High yields of plant extracts are extensively obtained by SFE using carbon dioxide as the solvent that is nontoxic, non-flammable,

Table 3: Regression coefficient model and P values for supercritical fluid extraction crude extraction yield by backward elimination.

Variables	Regression coefficient	P values*
Intercept	1.30	0.0002
A	1.27	<0.0001
B	-0.27	0.0681
AB	0.07	0.6602
A^2	0.92	0.0015
B^2	0.18	0.3479
A: Pressure, B: Temperature		
*P<0.05		

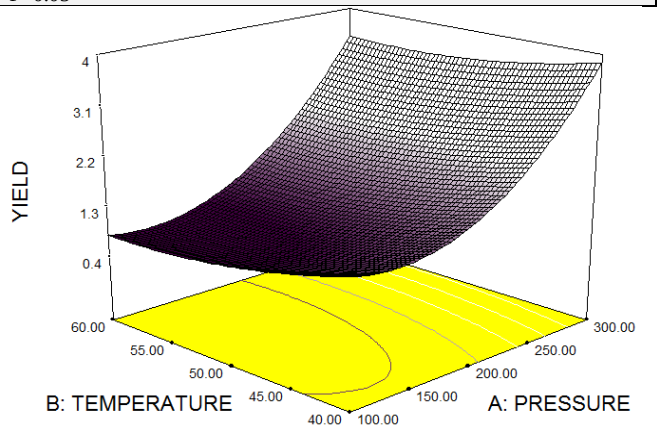


Fig. 3: Response surface plot of interaction between pressure (A) and temperature (B) on crude extraction yield.

Kar et al., 2009). For extraction parameters, the response surface cheap and available at a high degree of purity (Siddiq et al., 2010; indicated that an optimal point for CEY (3.59%) was obtained with pressure (A) and temperature (B) at 300 bar and 40°C, respectively. Further rise in temperature did not increase the CEY. However, the influence of temperature is a rather complex topic. As studied by Lepoyevic et al. (2017), rise in temperature could decrease the density of CO_2 which could lead to a decrease in solubility of the solute. However, increasing temperature would raise the vapour pressure of solute which contributes to potential safety hazard at atmospheric pressure. Hence, it is practical to lower the extraction temperature. From an economic point of view, the investment in energy to provide the desired temperature for extraction can be lessened. Siti Hafsah et al. (2016) also warned that the use of extraction temperature above 100°C could lead to thermal degradation of desired compounds in the extract and is inappropriate for long-term application of SC-CO_2. Menichini et al. (2011) have investigated the optimal conditions to extract volatile oil from Citrus medica L. cv with extraction pressure and temperature of 100 to 300 bar and 40°C to 60°C, respectively. The highest yield of targeted compounds (Citropten, 2,3-Dihydrobenzofuran and 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-Pyran4-one) was at the optimal condition of 40°C and 100 bar. Therefore, the optimal extracting temperature was selected at 40°C for this experimental study. On the other hand, the optimal extracting pressure was 300 bar. Although it is clearly evident that pressure increases the extraction yield greater than temperature, further study may be required to investigate the increment of CEY on increasing extracting pressure to increase the solubility of carbon dioxide in plants (Siti Hafsah et al., 2016). This case is also reported by Gopalan et al. (2000). However, it is not advisable to use very high extraction pressure as it could be a potential safety hazard due to high vapor pressure at room temperature (Mirofci, 2014).

The SC-CO_2 parameters were optimised using the numerical optimisation function of Design Expert. The combination of factors can maximise the crude extraction yield. The experiments using extraction conditions based on this optimal point were performed in order to confirm the predicted results of the optimised model. The predicted and experimental values of CEY obtained from Equation 2 are illustrated in Table 4. The statistical model was generated using the experimental values for the experimental parameters. The accuracy of the predicted value for CEY was 3.87% while the actual

Table 4: The difference between the experimental and predicted values for crude extraction recovery.

Description		Crude extraction yield (%)	
Pr (bar)	Temp (°C)	Experimental	Predicted
300	40	3.59	3.87

experimental result was 3.59%. Predicted and experimental values showed a good correlation which validated that the response model was suitable for the desired optimisation.

The efficiency of extraction yield depends on multiple extracting parameters such as pressure, temperature, solvent flow rate, extraction time (dynamic and static) and solvent polarity among others (Montgomery, 2001). The size of particles can also affect the crude/oil yield and extraction rate due to internal mass transfer limitations. Hence, to obtain high recovery of crude/oil, the plant needs to be ground prior to SC-CO_2 extraction. This is because smaller particles are able to increase the surface area for the solvent to solubilise into the structure and ruptures a large number of cell walls (Kar et al., 2009). The low yield of crude extract in this study could be attributed to the low ability of solvent channeling through the bed of ground *S.alata* leaves.

For a given pressure and temperature, the extraction yields may also increase in the presence of co-solvent (Mirofci, 2014). The added co-solvent may enhance the extraction yields as a result of interactions between the polar group and changes in local density, as well as an improvement for the cutbacks of supercritical fluid extraction carbon dioxide to extract polar components. Mirofci (2014) claimed that the yield is lower (only 1.5%) when using supercritical carbon dioxide without co-solvent. The addition of co-solvent methanol and ethanol leads to improvement of the extraction yield (5.56% and 5.54% respectively). This may occur due to the polarity of co-solvent that separates the polar compounds from materials effectively.

3.4. Biological Analysis

This study examined the hyaluronidase inhibiting potential and the total flavonoid content of dried *S.alata* which is widely known as a traditional cure for inflammatory disease and effective antioxidant activity. The hyaluronidase assay used to determine hyaluronidase inhibition activity is a simple standard that provide strong and reproducible activity validation (Sumantran et al., 2007). Dried *S.alata* was extracted with supercritical carbon dioxide at a flow rate of 35g/min. The parameters pressure (100 to 300 bar) and temperature (40˚C to 60˚C) were varied. In contrast, most of the biological activity studies on *S.alata* have been extracted by solvent extraction method such as methanol and ethanol, which is

super effective but endangere the environment (Marco et al., 1998; David & Seber, 1996; Pharkphoom et al., 2009). Hence, this study will be the first to investigate the hyaluronidase activity and total flavonoid contents of *S.alata* crude extract with recent extraction technology of environmentally-safe supercritical fluid extraction by carbon dioxide.

The results obtained are tabulated in Table 5. As shown in Table 5, *S.alata* showed a varying degrees of hyaluronidase inhibition activity (ranging from 7.08 – 41.19%) at final concentration of 100µg/mL. The most potent inhibitory activity was obtained at pressure and temperature of 200 bar and 60˚C, shown in Figure 4. The results clearly imparted that *S.alata* possesses noticeable hyaluronidase inhibition activity. It is known that hyaluronidase plays a crucial function in many biological systems, such as allergy and inflammation, by provoking the expression of anti- inflammatory genes, granulation of mast cells and release of chemical mediators (Nor Hayati et al., 2016; Sahasrabudhe & Deodhar, 2010). Further, inhibitory effect by potent inhibitors possesses great antiarthritic abilities (Sahasrabudhe & Deodhar, 2010). Since hyaluronic acid (HA) is naturally occurring in connective tissues, synovial fluid, umbilical cords and chicken combs, its degradation by hyaluronidase is linked to ophthalmic surgery for increase tissue permeability which is highly significant to speed up drug dispersion and delivery

Table 5: Hyaluronidase inhibition activity and flavonoid contents of *S.alata*.

Sample	Description		Hyaluronidase Inhibition Activity[a] (%)	Flavonoids content (% w/w)
	Pressure (bar)	Temperature (˚C)		
1	100	40	22.6105± 0.69	12.9904
2	100	50	7.6353 ± 0.28	44.9957
3	100	60	32.0633± 2.19	ND
4	200	40	7.0817 ± 2.75	26.1690
5	200	50	25.8434± 2.65	18.6384
6	200	60	41.1888± 2.39	12.9904
7	300	40	29.4107± 1.82	41.2304
8	300	50	29.4704± 2.40	24.2864
9	300	60	26.0324± 2.39	52.5263
ND= Not Determined				

[a]Values are expressed as mean inhibition (%) ± SEM of triplicate measurements from 3 independent experiments. Final concentration of tested samples in reaction mixture was fixed at 100µg/mL.

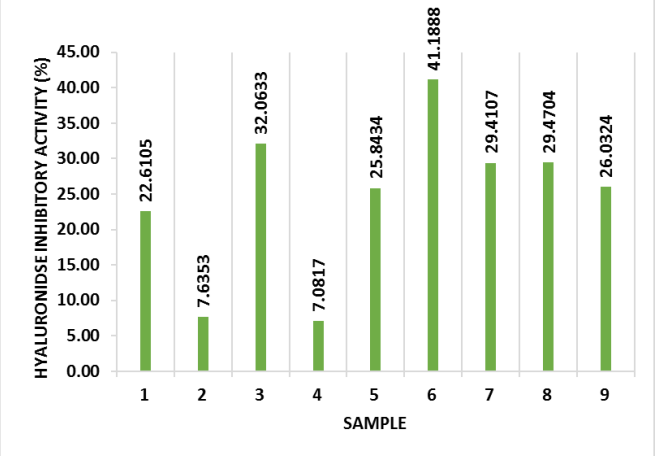


Fig. 4: Percentage of inhibitory hyaluronidase activity for all run using Hyaluronidase Assay.

(Necas et al., 2008). Since *S.alata* leaves shows inhibitory activity, these facts suggest that it may also offer a beneficial role in the management of allergies and inflammation, as therapeutic agents and application in ophthalmic surgery (Nor Hayati et al., 2016; Sahasrabudhe & Deodhar, 2010; Kuppusamy et al., 1990; Necas et al., 2008).

The performance of the assay was verified using apigenin as a reference. Apigenin is one of widely used flavonols, a class in the flavonoids that consist of 4',5,7-dihydroxyflavone backbone (Figure 5). Apigenin was used as a representative compound in the assay due to its potent inhibitory effect on hyaluronidase. In fact, this study proves that apigenin is a dependable reference as the inhibitory hyaluronidase activity was approximately 73.9% at a concentration about 500 mM. Kuppusamy et al. (1990) has also claimed similar results. They observed that apigenin is one of the most potent flavonoids amongst tannin, luteolin and kaempferol with an inhibition up to 66.5% at concentration of 250 µM. Further, its inhibitory effects are two-fold more potent that other corresponding glycosides namely apiin, quercetin and rutin. This is due to the double bond between carbons 2 and 3 as well as the hydroxyl substituents at positions 5, 7 and 4' on the chemical structure which allow high anti-peroxidative properties. Further, the total flavonoid contents of the crude SC-CO_2 extract were investigated.

Quantitative determination of total flavonoids was calculated on the basis standard of rutin and linearity of the calibration curve was achieved between 10 to 50 ppm concentration for rutin. ($y = 0.0215x - 0.0013$; $R^2 = 0.924$), shown in Figure 6. All extracts were investigated for flavonoids content except for Sample 3 (100 bar and 60˚C) due to insufficient amount of crude sample that was obtained from SFE. From Table 5, it can be observed that *S.alata* has temperature (60˚C) and pressure (300 bar) rather than at mild conditions. At fixed pressure of 200 bar, the total flavonoid contents were noticed to be decreased at increasing temperature, as shown favourable amount of flavonoid contents is obtained after extracting was found highest for Sample 9 at 300 bar and 60˚C (Figure 7). It

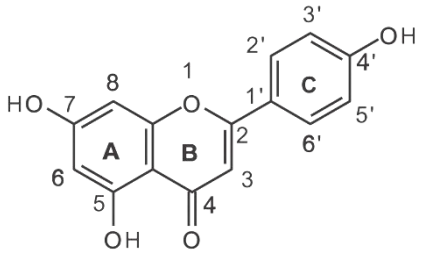


Fig. 5: Chemical structure of apigenin.

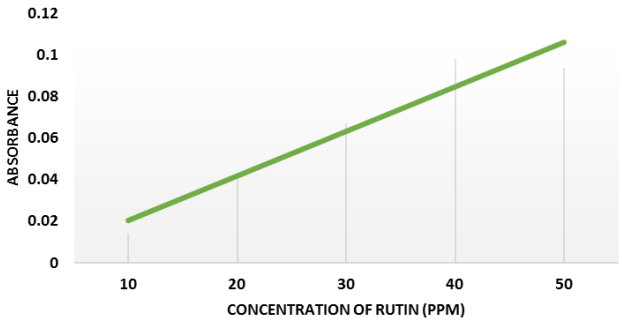


Fig. 6: Standard curve of rutin to determine total flavonoid contents of *S.alata* crude extract $y = 0.0215x - 0.0013$; $R^2 = 0.924$.

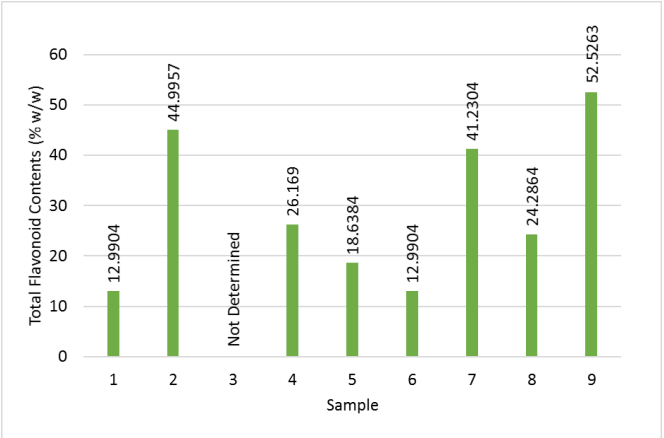


Fig. 7: Percentage of total flavonoid content for all samples.

shows that flavonoids were preferable to be extracted at high with supercritical fluid carbon dioxide. The concentration of flavonoids throughout Sample 4 to Sample 6. It can be observed similar trend for Sample 7 and 8, at 40 and 50˚C respectively at fixed pressure of 300 bar. However, the contents had increased at 60 ˚C. This may be due to increased selectivity at high temperature and pressure (Liu et al., 2014). This proves that SC-CO_2 extraction method is a viable and practical procedure for flavonoid compounds extraction. This can also open up new opportunities for supercritical extraction to isolate many other valuable compounds from plants that may be used in cosmetics, pharmaceutical and dermatological fields.

Currently, many researchers have shifted to using SFE rather than using solvents for extraction because SFE uses mild processing conditions, is readily separated from the solutes and recognized as safe by FDA and EFSA (Karale et al., 2011; Suetsugu et al., 2013). Hence, this make supercritical fluid a promising method to isolate flavonoid compounds from any type of plants. Flavonoids constitute an excellent antioxidant activity and thus, *S.alata* SFE crude extract makes a potential alternative for cosmetics as well as pharmaceutical industry due to diverse bioactivity such as preventing oxidant of low density lipoprotein and inhibit peroxidation of lipid (Rahman et al., 2008; Formica & Regelson, 1995). Moreover, presence of the phenolic hydroxyl groups enable makes them a potent antioxidant that are able to scavenge the reactive oxygen species effectively (Cao et al., 1997). Further investigation of antioxidant activity of *S.alata* SFE crude extract can be determined by free radical scavenging DPPH procedures.

These preliminary results suggest that *S.alata* may possesses some anti-inflammatory properties through the inhibition of hyaluronidase. However, the moderate hyaluronidase inhibitory result from *S.alata* requires further investigation and optimization of supercritical fluid extraction parameters to enhance the inhibition activity and the extraction of valuable compounds without degrading the plant material and are economically efficient.

4. Conclusion

In this study, the optimum extraction conditions for supercritical fluid extraction to yield crude extract from *S.alata* dried leaves were not yet determined. According to the CCD and response surface analysis for SFE method, a quadratic polynomial model was used to model the yield of crude extract from a fixed mass of dried leaves (130 g) and a fixed CO_2 flow rate (35 g/min). The two independent variables involved in the prediction were pressure and temperature. The results indicated that the extraction pressure had the greatest impact on oil yield within the range of the operating conditions investigated. The highest yield was at the pressure of 300 bar and temperature of 60˚C, achieving a crude extract of 3.62%. The results show that SFE was effective to obtain crude from *S.alata*. The biological analysis include hyaluronidase inhibition activity and total flavonoids content was successfully obtained for *S.alata* crude extract. The highest value obtained for *S.alata* for hyaluronidase inhibition activity was 41.19% (at P: 200 bar and T: 60˚C) while for total flavonoids content was 52.53% w/w (at P: 300 bar and T: 60˚C). Therefore, *S.alata* crude extract obtained with SC-CO_2 may have potential for use as an anti-inflammation and antioxidant component in various dermatological and cosmetic industries. The application of SC-CO_2 as a safe solvent that can minimize wastewater compared to conventional techniques which uses organic solvents for extracting of oils from natural sources such methanol and ethanol. This method was effective in crude extraction yield and hence made supercritical fluid technology as an alternative technique for the extraction of pure and high quality crude from *S.alata*. It is a cost effective technique for laboratory scale and it seems to be appropriate for industrial crude/oil extraction. However, further study should be investigated for the optimization of both parameters pressure and temperature on the yield extract.

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