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Bioactive Components from Flowers of *Sesbania grandiflora L.*: Extraction and Optimization Studies

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Abstract : Plant based extracts are attractive sources of nutraceutical which have been shown to produce promising results in the treatment of curing many diseases and disorders. Sesbania grandiflora Linn is one such plant commonly known as Agathi belongs to the family, Fabaceae native to tropical region that has been used medicinally for centuries. The present study deals with bioactive components extraction from the flowers of Sesbania grandiflora using different solvents namely ethanol, methanol, petroleum ether, acetone, and n-hexane using soxhlet apparatus. Response surface methodology is applied to optimize the extraction process of bioactive components from Sesbania grandiflora flower. The effect of process parameters such as temperature (40 ^{0}C to 60^{0}C), time (8hr to 10hr) and the quantity of sample (10gm to 20gm) on extraction yield was studied. Among the five solvents used in the study, a maximum yield 9.95% was found when ethanol is used. Phytochemical constituents of the extract were analyzed using phytochemical screening methods. The extraction yield is optimized for different process variables using design of experiments.

Key words : Sesbania grandiflora, Extraction, Soxhlet, Response surface methodology.

Introduction

Since ancient age nature has been a source of medicinal agents and many of the traditional drugs have been isolated from natural source. Research focus on the natural source has been increased recently due to its minimum side effects. Articles reveal that almost more than 15000 plants have been used by different ethnic communities in India. Many active compounds have been isolated from the plants through various extraction method using different solvents and these are pharmacologically active[6].

Even today, majority of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparation of Ayurveda medicines. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity. In some cases, the crude extract of medicinal plants may be used as medicaments. On the other hand, the isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance[4]. In present scenario, utilization of the herbal plants for physical and mental ailments is in an uphillmovement.

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Sesbania grandiflora is a small, loosely branching tree that grows up to 8-15 m tall and 25-30 cm in diameter; roots normally heavily nodulated with large nodules; the tree can develop floating roots. Leaves alternate and compound; pinnate, 15-30 cm long with 12-20 pairs of oblong, rounded leaflets, 3-4 cm long and about 1 cm wide; leaves borne only on terminal ends of branches; leaves turn bright yellow before shedding. Flower clusters hanging at leaf base have 2-5 large or giant flowers; pink, red or white, pea like, 5-10 cm in length, curved about 3 cm wide before opening. Pods long and narrow, hanging down 30-50 cm by 8 mm; septate, wide, flat, with swollen margins and about 15-40 pale-coloured seeds; seed is beanlike, elliptical, red brown, 6-8 in a pod, 3.5 mm, each weighting 1g[5]. The present research is mainly concentrated on flower of sesbania grandiflora due to its special pharmacological activities.

100g of Sesbania grandiflora flower contains 91.58g water, 27 kcal, 113 kJ, 1.28 g protein, 0.04g fat, 0.38gash, 6.73g carbohydrates, 18mg calcium, 0.84mg iron,12mg magnesium, 30mg phosphorous , 184mgpotassium, 15mg sodium, 0.8µg selenium, 73mg vitamin C, 0.083mg thiamin, 0.081mg riboflavin and 102µg folate according to the USDA nutrient database[2].

Each part of the plant has its unique therapeutic efficacy against different disorders which is given in table 1.

Table 1 Pharmacological Activities of different parts of the plant

Parts	Pharmacological Activities
Bark	Anti ulcerogenic and Anticonvulsant
Leaf	Anxiolytic, Hepatoprotective, antioxidant and Antiurolithiatic activity.
Flower	Anti-cancer, Anti-microbial Analgesic and Anti pyretic activity.
Fruit	Anemiaand Bronchitis.
Root	Anti-inflammatory and Anti pyretic.

To determine the pharmacological activity and phytochemical screening the plant extract is extracted using different solvents. For this present investigation on extraction of bioactive from sesbaniagrandiflora flower, soxhlet extraction process with different solvents is used due to its maximum removal of bioactive components and the process is easy and cost effective. The maximum yield was found out among the extraction solvents and with that solvent the extraction is optimized using different process variables such as time, temperature and quantity of sample using design of experiment (response surface methodology).

Materials and Methods

Plant material

Sesbania grandiflora flowers were collected from a place named Otthakalmandapam near Coimbatore, Tamilnadu, India.

Plant sample preparation

The plant material collected was dried in the shade and finely powdered so that the rate of extractionincreases.

Extraction method

10g of dried powder of *C. quadrangularis* stem was extracted in 100 ml of solvents such as ethanol, methanol, petroleum ether, acetone, and n-hexane using a Soxhlet apparatus at a temperature of 40^0 C for 8 hrs. Then the extract was concentrated and freed of solvent using simple distillation to dryness. The dried crude concentrated extract was weighed to calculate the extraction yield. The yield was calculated using the formula

$$\% \text{ Yield} = (W_2 - W_1)/W_3 \times 100$$

Where,

W_1 = weight of the extraction cup
W_2 = weight of the extraction cup + extract
W_3 = weight of the sample

Design of experiments

The use of design of experiments (DOE) in extraction of plant material will play a central role in the development and modernization of processes for preparations of standardized phytotherapics. The design of experiments methodology offers the maximum return in terms of information about the interplay of multiple factors while requiring the minimum investment.[3]. The main objective of DOE is to minimize the trial runs and make the work easy with minimum number of experimental trials [1].

The objective of the present work was to apply response surface methodology to optimize the yield for extraction of antioxidant from the raw material. Several important factors, such as extraction time, and extraction temperature and quantity of sample were systemically analyzed using a response surface methodology (Central composite design).

Optimization

Among the five solvents used in the study, a maximum yield 9.95% was found when ethanol is used as extraction solvent. The values are given in table 1 and graph is given in table 2. The extraction yield is optimized for different process variables - temperature (40 ^{0}C to 60^{0}C), time (8hr to 10hr) and the quantity of sample (10gm to 20gm) using design of experiments(RSM-CCD).

Phytochemical screening

Phytochemical screening was done according to the standard procedure[4].

Results and Discussion

The extraction was carried out using soxhlet apparatus with different solvents such ethanol, methanol, hexane, acetone, petroleum ether at a temperature of 40^0 C for 8hr. The yield was given in table 2 and figure 1.

Table 2 Yield of the samples extracted at a temperature of 40^{0}C for 8 hrs for different solvents

Extract	% Yield
Ethanol extracts (EE)	9.97%
Methanol extract (ME)	8.78%
Acetone extract (AE)	2.64%
Hexane extract (HE)	1.99%
Petroleum extract (PE)	0.69%

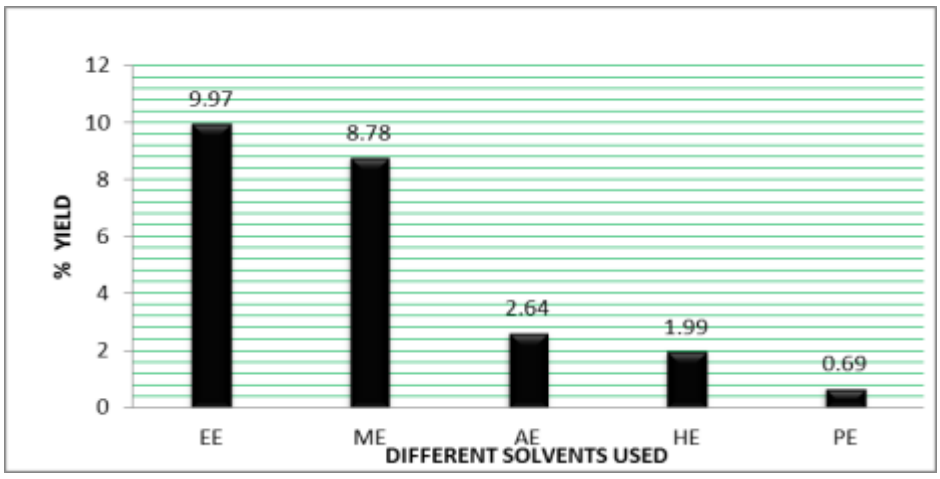

Figure 1 Yield of different solvents

It was observed from the figure 1 that the maximum yield was found when ethanol is used as a solvent at a temperature of 40^{0}C for 8hrs and the corresponding yield was found to be 9.97%.

Selection of solvent

In the table 2, it can be observed that the extraction efficiency order in the sequence of descending was: ethanol methanol>acetone>n-hexane>petroleum ether. It was decided to employ ethanol as solvent for the development of the extraction method. Because it had a high extraction efficiency (9.95%) and relatively high selection rate and it is an economic and green solvent compared with methanol andacetone.

Response Surface Methodology

Therefore, three selected factors and their levels applied in this study are decided.

They were numbered and listed in Table 3 for CCD.

Table 3 Factors and their levels for the experiments

Factor	Level 1	Level 2	Level 3
(A)Extraction temperature(℃)	40	50	60
(B)Extraction time (hrs.)	8	9	10
(C)Quantity of sample(g)	10	15	20

Up to now, all preparation work was done; the next step is to design the experiment using RSM. According to the CCD principle, the experiment arrangement was designed. It was shown in table 4 together with the experimental results.

Table 4 RSM – CCD (levels of three different factors and obtained results)

Experiment number	A	B	C	Yield (%)
1	60	10	10	8.8
2	60	8	20	10.6
3	40	10	20	13.9
4	40	8	10	9.9
5	35.86	9	15	6.93
6	64.14	9	15	7.82
7	50	7.59	15	14.2
8	50	10.41	15	15.6
9	50	9	7.93	12.6
10	50	9	22.07	18.4
11	50	9	15	16.6
12	50	9	15	16.6
13	50	9	15	16.6
14	50	9	15	16.6
15	50	9	15	16.6

Statistical analysis

To optimize the extraction method with a highest yield, a Response Surface Methodology (RSM) optimization was conducted. The independent variables and response values shown in Table 4 were used to generate a mathematical model that expresses the behavior of the experiment. This model allows the assessment of predicted response (Y) as a function of the independent variables and their interactions. The calculated model in terms of coded factors is generated by RSM for the response in this study is shown in equation 1.

$$Y = 1.24 + 0.019*A + 0.014*B + 0.058*C + 1.090E - 0.03*A*B - 2.201E - 0.03A*C +$$

$$0.061*B*C - 0.18*A^2 - 0.026*B^2 - 0.021*C^2 \ldots\ldots (1)$$

where Y is yield of extract ; A, B and C are Temperature, Time and Quantity of sample respectively

Table 5 ANOVA table for the design in RSM – CCD

ANOVA for Response Surface Quadratic Model

Analysis of variance table [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	231.49	9	25.72	165.15	< 0.0001	significant
A-Temperature	*0.41*	*1*	*0.41*	*2.60*	*0.1678*	
B-Time	*0.98*	*1*	*0.98*	*6.29*	*0.0539*	
C-Quantity of sa	*16.82*	*1*	*16.82*	*108.00*	*0.0001*	
AB	*0.72*	*1*	*0.72*	*4.63*	*0.0840*	
AC	*6.056E-003*	*1*	*6.056E-003*	*0.039*	*0.8514*	
BC	*4.02*	*1*	*4.02*	*25.83*	*0.0038*	
A^2	*188.03*	*1*	*188.03*	*1207.33*	*< 0.0001*	
B^2	*10.42*	*1*	*10.42*	*66.89*	*0.0004*	
C^2	*5.73*	*1*	*5.73*	*36.81*	*0.0018*	
Residual	0.78	5	0.16			
Lack of Fit	*0.78*	*1*	*0.78*			
Pure Error	*0.000*	*4*	*0.000*			
Cor Total	232.26	14				

To assess the goodness of the fit of the model that has been generated by the RSM – CCD, the ANOVA was conducted and shown in table 5. The probability of error P was 0.011, R^2 was 0.9921, adjusted R^2 was 0.9780, adequate precision (AP) was 24.277, standard deviation (SD) 0.21, and coefficient of variation (CV) 1.92%. Based on the result, it is shown that the model is good enough to express the interaction of the independent variables and their responses. The calculated R^2 values are desirably high (close to 1). The predicted R^2 value is also in agreement with the adjusted R^2value. In addition, the AP value for the response is greater than 4, which is desirable. It implies that all of the predicted models could be used to navigate the design space defined by the RSM - CCD. Furthermore, the result indicated that the model is considered reproducible. This is due to the value of CV, which is the error expressed as a percentage of the mean, being less than 10%.

Response plot

The response plot for the various process variables are given in 3D graph with one variable as actual factor. The graphs are shown in figure 2, 3 and 4.

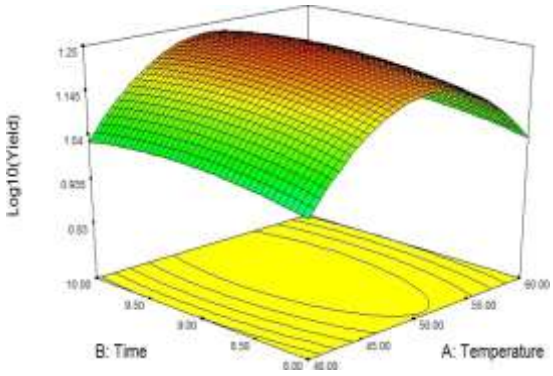

Figure2

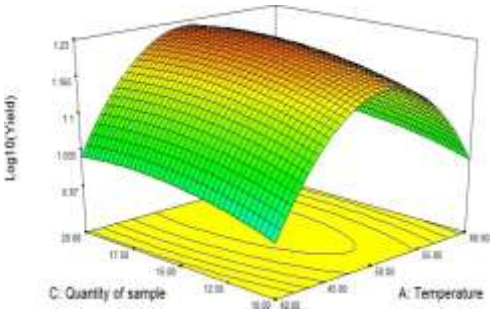

Figure3

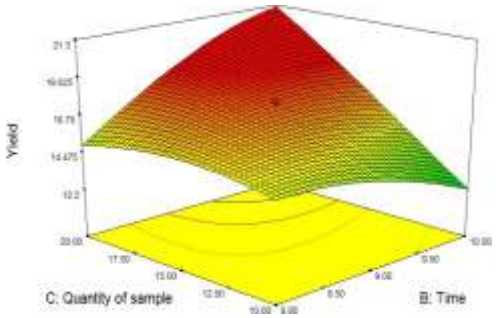

Figure 4 Response plot for temperature and time, temperature and quantity of sample and time and quantity of sample

Effect of different process variables on yield

When temperature and time increases the extraction rate increases to a certain limit till 50^0 C at 9 hrs and at higher temperature 60^0 C at 10 hrs. the extraction rate decreases due to thermal degradation and at lower temperature the extraction rate decreases due to insufficient time and temperature. The figure 5 shows the effect of temperature and time (1,2,3,4… 15 represents the temperature and time in table4).

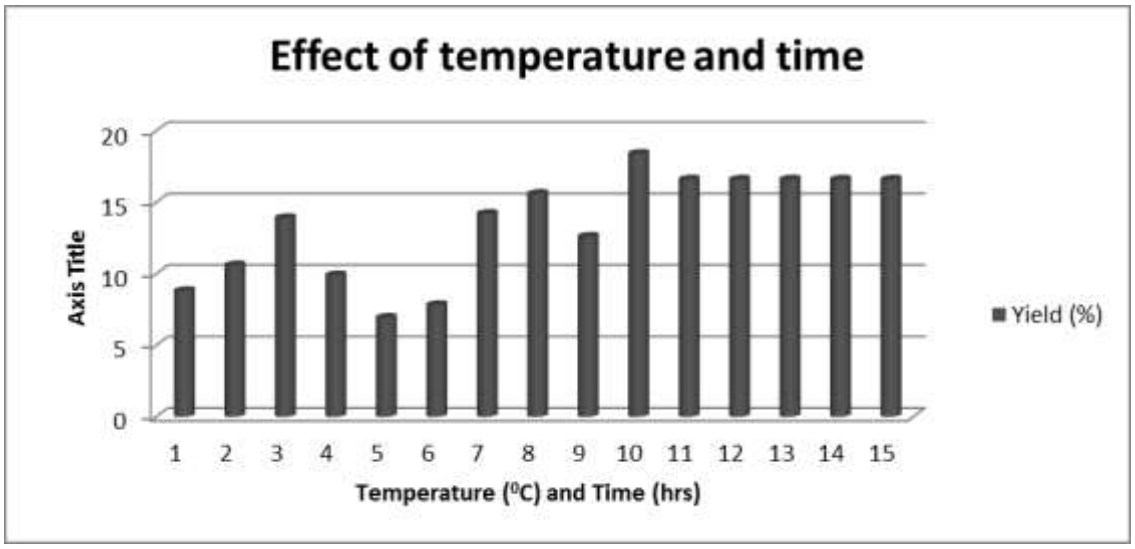

Figure 5 Effect of temperature and time

It was observed that when the quantity of sample and decreases the extraction rate decreases and the graph was plotted and shown in figure 6 (1,2,3,4 .....15 represents the temperature and time in table 4).

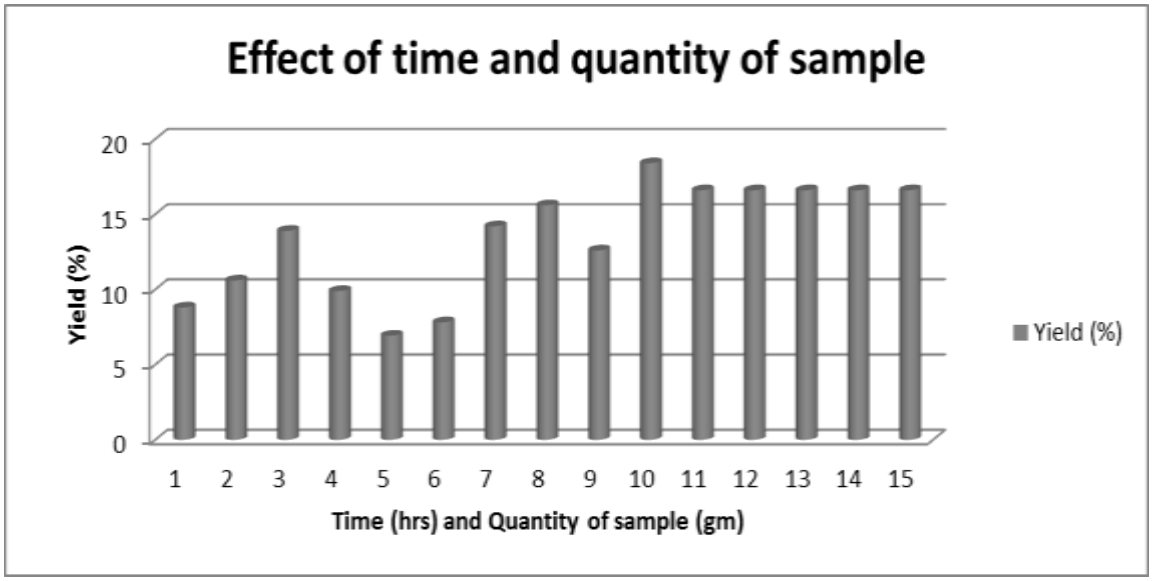

Figure 6 Effect of time and quantity of sample

It is observed that when the temperature and sample quantity increases the extract yield increases and the effect of temperature and quantity of sample is shown in the figure7(1,2,3,4… 15 represents the temperature and time in table4).

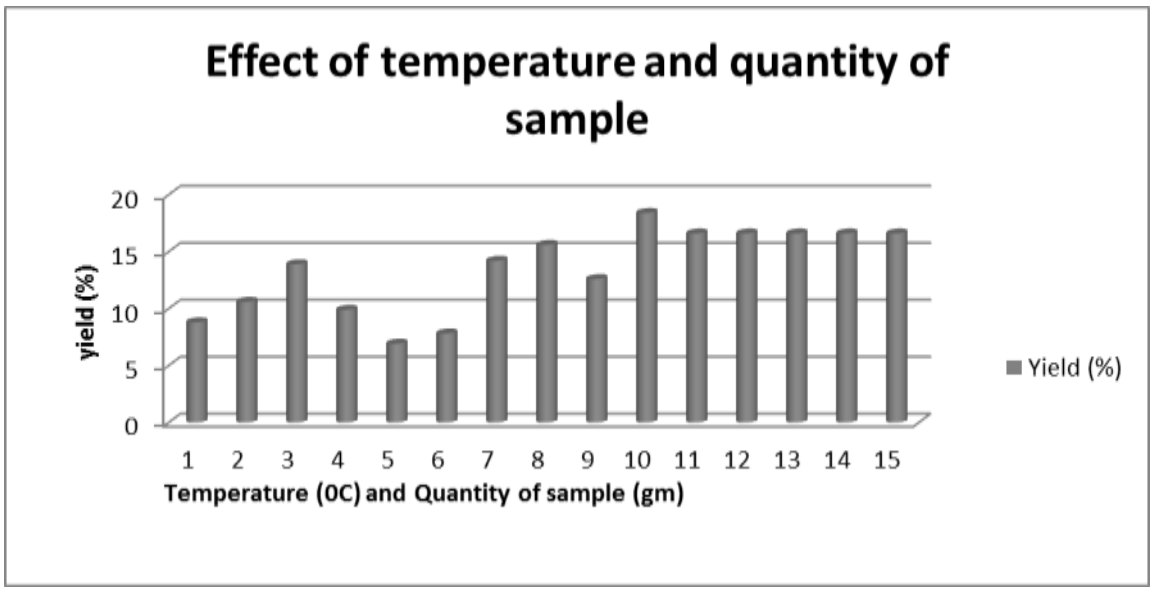

Figure 7 Effect of temperature and quantity of sample Phytochemical screening

Phytochemical screening was done using standard procedure and the results were given in table 6

Table 6 Phytochemical screening for ethanol extract

Phytochemical Constituents	Ethanol Extract
Alkaloids	(+)
Carbohydrates	(+)
Glycosides	(+)
Flavonoids	(+)
Phytosterols	(+)
Fixed oil and Fats	(-)
Saponins	(-)
Phenolic and Tannins	(+)
Lignin's	(+)
Protein and Amino Acids	(+)
Gums and Mucilage	(-)

(+) – Presence, (-) – Absence

The results of RSM with three factors – temperature, time and quantity of sample are given in Table 3. Fifteen runs were carried out to cover all possible combination of the two factors. The results are expressed as the yield of crude extract. The highest yield was found to be 18.4% at a temperature of 50^0 C for 9hr with 22.07 Gms of sample and the lowest yield was found to be 6.93% at a temperature of 35.86^0 C for 9hr with 15 Gms of sample when using ethanol as extraction in soxhlet apparatus.

Conclusion

Conventional soxhlet extraction has been the most used extraction technique worldwide for a number of decades, surpassing the performance of other extraction alternatives and being used as an efficiency reference for the comparison of its conventional and new counterparts. Soxhlet extraction is an efficient method for extraction of bioactive components which is comparatively economical and easy for industrial process. Firstly, through single factor experiments, ethanol was chosen as the extraction solvent; levels of different factors for design of experiment were also determined. Next to validate the optimum process condition generated by RSM - CCD; a laboratory test was conducted at the selected optimum condition (see Table 3). Finally, the best parameters for soxhlet was determined to be 50℃(extraction temperature), 9 hrs. (extraction time), 22.07g(quantity of sample). Under this condition the extract yield was found to be 18.4%. The results show that Response Surface Methodology is an effective way to optimize conditions when several factors affect the experiment. The extract is analyzed for its phytochemical components and the presence of components such as Alkaloids, Carbohydrates, Glycosides, Flavonoids, Phytosterols, Proteins and Amino acids, Phenolic and Tannins, Lignin's aredetermined.

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