OPTIMIZATION PROCESS FOR INCREASING THE YIELD OF CRUDE ALKALOID FROM *Litsea polyantha* JUSS. BARK

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ABSTRACT

Litsea polyantha Juss. (Lauraceae) also known as Litsea monopetala Roxb. is a small to medium sized evergreen tree distributed chiefly in tropical and subtropical Asia, Australia and the Pacific Islands. The bark of Litsea polyantha Juss. has a long history of medicinal use among the traditional healers of Oraon and Munda community of Jharkhand (India). In this work, optimization process for increasing the yield of crude alkaloid from Litsea polyantha Juss. bark was performed by taking pH and temperature as the factors influencing the yield. With the help of yield optimization by 2^2 factorial design the percentage yield of crude alkaloid was increased from 3.8% w/w to 17.3% w/w.

Keywords: factorial design; *Litsea polyantha*; alkaloids; plant extracts; ethnopharmacology.

1. Introduction

India is one of the world's 12 leading biodiversity centers having 45,000 different plant species. Out of these, about 7,000-7,500 plants are being used for their medicinal values [1]. The use of herbal drugs as complementary medicine is prevalent and gaining world wide popularity. Many drugs are derived directly from plants; while the others are chemically modified natural products [2]. *Litsea polyantha* Juss. (Lauraceae) also known as *Litsea monopetala* Roxb. is a small to medium sized evergreen tree. The bark of *L. polyantha* Juss. has a long history of medicinal use among the traditional healers of Oraon and Munda community of Jharkhand. It is known by the popular names of Pojo, Kakuri, Munga and Barkukuchita. The bark of *L. polyantha* Juss. is mildly astringent and is reported to be used for diarrhea. Powdered bark and roots are used for pains, bruises and contusions and for fractures in animals. Previous chemical investigation of this plant has revealed the presence of beta-sitosterol and actinodaphnine in the bark, an arabinoxylan containing D-xylose and L-arabinose in a molar ratio of 1:2 in leaves and chalcones & eugenol in the bark [3]. In this work, optimization process for increasing the yield of crude alkaloid from *Litsea polyantha* Juss. bark was performed by taking pH and temperature as the factors influencing the yield.

2. Material and methods

2.1 Plant material

The bark of *L. polyantha* Juss. was collected from BIT, Mesra of Ranchi with the help of tribal. The bark was authenticated and the voucher specimen (BIT 417; 2008-09) was preserved in the Department of Pharmaceutical Sciences, BIT, Mesra.

2.2 Extract Preparation

Harbone's method was used to isolate crude alkaloid from the plant. MELP was extracted with 10% v/v acetic acid and was allowed to stand for at least 4 h. The extract was concentrated to one-quarter of the original volume and the crude alkaloid was precipitated by drop wise addition of concentrated NH_4OH. The alkaloid was collect by centrifugation, washed with 1% w/v NH_4OH and then dissolved in chloroform. The percentage yield of crude alkaloid was around 4% w/w. To increase the percentage yield of crude alkaloid, the Harbone's method was modified using 2^2 Factorial Design, taking pH and temperature as the factors influencing the yield.

2.3 2^2 Factorial Design Method

Isolation of alkaloid was carried out by taking pH and temperature as the factors influencing the yield. The initial pH of the extract was 6 and the isolation of crude alkaloid was carried out in the room temperature (30 °C). The percentage yield of crude alkaloid was 3.8%. In the factorial design the initial experiments were carried out at four different levels of pH and temperature viz. pH 5 and temperature 20 °C; pH 5 and Temperature 40 °C; pH 7 and temperature 20 °C and pH 7 and temperature 40 °C.

2.4 Statistical Analysis

The statistical analysis is carried out with help of ANOVA. The Null hypothesis is taken that there is no significant change on the overall experiment on either changing the pH or temperature or both. Also, there is no interaction between pH and temperature. The sources of variation are A: pH, B: temperature, AB: interaction between pH & temperature and Errors. The sums of squares are calculated by the following equations:

$$SS_A = \frac{[ab + a - b - (l)]^2}{4n} \qquad SS_B = \frac{[ab + b - a - (l)]^2}{4n}$$

$$SS_{AB} = \frac{[ab + (l) - a - b]^2}{4n} \qquad SS_T = \sum_{i=1}^{n}\sum_{j=1}^{n}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{4n}$$

$$SS_E = SS_T - SS_A - SS_B - SS_{AB}$$

Where, SS_A: sums of squares pH; SS_B: sums of squares temperature
SS_{AB}: sums of squares interaction; SS_E: sums of squares of errors
SS_T: sums of squares total

3. Results and Discussion

The result of the 2^2 Factorial Design represented in Table 1 indicates that better yield is obtained if the pH is at low level and the temperature is at high level. At pH 5 and 40 °C the percentage yield of crude alkaloid was 7.9% w/w, which is twice of the initial yield of crude alkaloid at pH 6 and 30 °C (3.8% w/w). The Main effect of A (pH) was negative. Increasing pH from the low level to high level decreased the yield. The Main effect of B (temperature) was positive. Increasing temperature from the low level to high level increased the yield. There was some negative interaction between A and B, but its effect appears to be smaller relative to the main effects of A & B.

Statistical analysis using ANOVA for the factorial experiment is represented in Table 2. By comparing the calculated F values with the tabulated F values it was observed that both pH and temperature has significant effect on the yield of alkaloid and there is no significant interaction between pH and temperature at any level.

Table 1: 2^2 Factorial Design to increase the percentage yield (w/w) of crude alkaloid from *Litsea polyantha* Juss. bark

Treatments	Factors		Treatment Combination				Replicates % yield			Sum	Average % yield (w/w)
	A	B					I (i)	II (j)	III (k)		
l	-	-	A Low	5	B Low	20	5.2	4.6	6.1	15.9	5.3
a	+	-	A High	7	B Low	20	3.1	4.5	2.8	10.4	3.5
b	-	+	**A Low**	**5**	**B High**	**40**	8.9	7.2	7.6	23.7	**7.9**
ab	+	+	A High	7	B High	40	4.2	3.9	5.1	13.2	4.4
B at High Level and A at Low Level gives Better Yield											

Where, A = pH and B = Temperature (°C); a: Represents treatment combination of A at High Level & B at Low Level; b: Represents treatment combination of B at High Level & A at Low Level; ab: Represents treatment combination of both A & B at High Level; 1: Represents treatment combination of both A & B at Low Level

Table 2: ANOVA of factorial experiment for increasing the percentage yield (w/w) of crude alkaloid from *Litsea polyantha* Juss. bark

Sources of Variation	**Sum of Squares**	**Degree of Freedom**	**Mean Square**	**F0**
A	SS_A = 21.33	1	21.33	33.16
B	SS_B = 9.36	1	9.36	14.55
AB	SS_{AB} = 2.08	1	2.08	3.24
ERROR	SS_E = 5.15	8	0.64	
TOTAL	SS_T = 37.92	11		**F 0.05 (1,8) = 5.32**

SS: Sum of Squares; A: pH; B: Temperature; AB: Interaction between pH & Temperature

It was obtained that by decreasing the pH (5) and increasing the temperature (40 °C) from the starting pH (6) and temperature (30 °C), the percentage yield of the crude alkaloid increases. Therefore, for the next step of optimization of the percentage yield by factorial design, pH 5 and 40 °C temperature was considered as the midpoint. *Regarding the yield optimization by* 2^2 *factorial design*, the result once again indicates that better yield is obtained if the pH is kept at low level and the temperature at high level. At pH 4 and 50 °C the percentage yield of crude alkaloid was 12.8% w/w, at pH 3 and 60 °C the percentage yield of crude alkaloid was 15.4% w/w and at pH 2 and 70 °C the percentage yield of crude alkaloid was 17.3% w/w. With the help of yield optimization by 2^2 Factorial Design the percentage yield of crude alkaloid was increased from 3.8% w/w to 17.3% w/w. The Main effect of A (pH) in all of the optimization steps was negative. Increasing pH from the low level to high level decreased the yield. The Main effect of B (temperature) was positive. Increasing temperature from the low level to high level increased the percentage yield. Some negative interaction has been seen between A and B, but its effect appears to be smaller relative to the main effects of A & B. The response surface data shows the average percentage yield of the crude alkaloid at different pH and temperatures. Figure 1 of 3D response surface suggests that any pH and temperature within the black response surface (top of the graph) will lead to a high yield of the crude alkaloid.

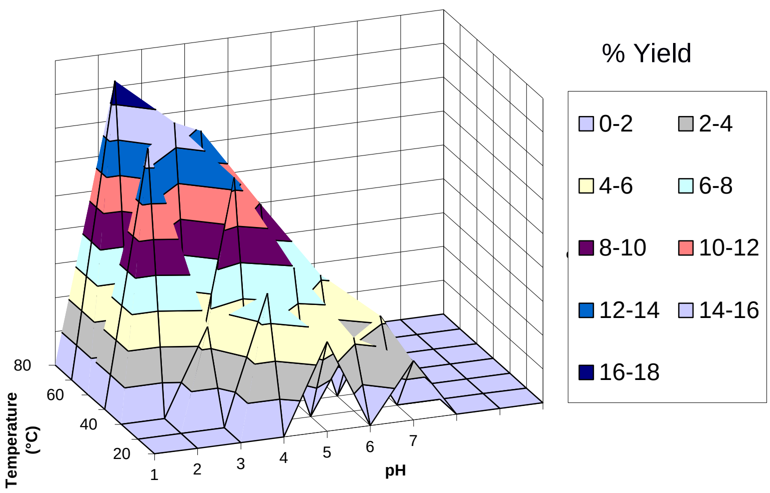

Figure 1: 3D Response Surface graph showing average percentage yield (w/w) at different pH and temperatures

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