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Extraction Condition of *Echinocactus grusonii*

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***Abstract*—**The optimal extraction condition of dried *Echinocactus grusonii* powder was studied. The three independent variables are raw material drying temperature, extraction temperature, and extraction time. The dependent variables are both yield percentage of crude extract and total phenolic quantification as gallic acid equivalent in crude extract. The experimental design was based on central composite design. Highest yield percentage of crude extract could get from extraction condition at raw material drying temperature at 60˚C, extraction temperature at 15˚C, and extraction time for 25 min ˚C**.** Moreover, the crude extract with highest phenolic occurred by extraction condition of raw material drying temperature at 60˚C, extraction temperature at 35 ˚C, and extraction lasting 25 min.

***Keywords*—**Drying temperature, Extraction temperature, Optimal condition, Total phenolic

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I. INTRODUCTION

OVERWEIGHT and obesity among people are generally defined as 'a weight that is greater than what is healthy for a specific height'[1]. Obesity is associated with several medical problems such as diabetes mellitis, gallbladder disease, osteoarthritis, heart disease and some forms of cancer [2] and is a major health problem in both developing and developed countries.

In 1963, the National Food Research Institute, CSIR, Pretoria, South Africa, investigated more than a thousand species of indigenous plants that are used as food, among them *Hoodia* Sweet ex Decne. species. In a reactivation of the project motivated by Prof P.S. Steyn in 1986, *Hoodia* species was investigated at the National Chemical Research Laboratory, CSIR, Pretoria, South Africa. In an initial experiment on mice meant to test the thirst quenching properties as reported by Pappe (1862)[3] and Laidler (1928)[4], the appetite-suppressant activity of the extracts was observed. This has led to further research and development at the CSIR, the identification of an active compound, a patent application [5], licensing of the patent to international companies and entering into a benefit-sharing agreement with a Khoisan community.

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The prospect of a safe, natural appetite-suppressant agent has sparked considerable interest among the general population and commercial companies worldwide. Currently, there are more than 20 international patent applications/registrations on *Hoodia gordonii*, and many *Hoodia*-containing commercial preparations available on the market. A natural anti-obesity agent from *Hoodia* promises to become one of the few major products based on plants indigenous to the African Continent.

Hoodia gordonii is widely distributed through the arid areas of South Africa and Namibia. In common with other *Hoodia* species, it is a multi-stemmed succulent with thick, erect, cylindrical, fleshy and fairly hard, glabrous, grey-green to grey-brown stems [6]The tubercles of *Hoodia gordonii* are prominent, fused in their lower halves into 11–17 obtuse angles along stem, each tipped with a sharp spine 6–12 mm long [6]. As a result of the spiny nature of *Hoodia gordonii*, it is often referred to as 'cactus' or 'desert cactus' in the popular press although it is not at all related to a true cactus, which belongs to the *Cactaceae* family.

In South Africa, *Hoodia* species are protected and permits are required from the authorities for the collection, cultivation, transport or exporting of the plants. Being a desert succulent, *Hoodia gordonii* is a slow-growing plant and cultivation is not easy. However, the limited number of plants available in the wild cannot sustain a commercial market and future commercialization will strongly depend on cultivated plants.

From previously described, attention paid to study *Echinocactus grusonii* which is the plant in the family of *Cactaceae* originated in Mexico smae as Hoodia gordonii but cultivated in Thailand. *Echinocactus grusonii* is a well known species of cactus native to central Mexico from San Luis Potosi to Hidalgo. Described by Heinrich Hildmann in 1891[7], it is popularly known as the Golden Barrel Cactus, Golden Ball or, amusingly, Mother-in-Law's Cushion. It belongs to the small genus *Echinocactus*, which together with the related genus *Ferocactus*, are commonly referred to as barrel cacti.

Despite being one of the most popular cacti in cultivation, it is rare and critically endangered in the wild.

Growing as a large roughly spherical globe, it may eventually reach over a meter in height after many years. There may be up to 35 pronounced ribs in mature plants, though they are not evident in young plants, which may have a knobbly appearance. Note: Younger Golden Barrels do not look similar to the mature ones. The sharp spines are long, straight or slightly curved, and various shades of yellow or, occasionally, white. Small yellow flowers appear in summer around the crown of the plant, but only after twenty years or so.

Widely cultivated in warmer climates around the world, it is considered easy to grow and relatively fast growing. It has been increasingly used as an architectural plant in garden design. Jamie Durie, one of Australia's leading garden designers, has called it one of his personal favorite plants. A white-spine form is also in cultivation, as is a short-spine form.

While easy to grow, these plants do have some basic requirements; an average minimum winter temperature of 12°C, good drainage with less watering in winter. Excess water in cool periods may lead to rot. Golden Barrels are hardy to about 15ºF (-8ºC) for brief periods.

Outside Mexico, *Echinocactus grusonii* specimens may be seen in collections of desert plants in many botanical gardens[7-11].

For the optimal extraction condition, many method could be applied to get the optimum condition for correlation between independent and dependent variables [12-15]. The independent variables for bioactive extraction are raw material drying temperature, extraction temperature, extraction time, ratio of solvent to raw material etc. Also, dependent variables might be the yield percentage of crude extract, quantity of bioactive compounds, and others.

The study follows by focus on chemical composition and optimal condition of extraction to get highest total phenolic compound including yield percentage.

II. Materials and Method

A. Sample preparation

The *Echinocactus grusonii* from Khonkaen Province was used as raw material. The plants was trimmed and clean, chopped and tray drying at temperature at 50, 60, 70, 80 และ 90˚C. Dry powder was prepared by ground dry plants with hammer mill then pack in vacuum package kept in desiccators until use.

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B. Proximate analysis

Dry *Echinocactus grusonii* powder was analysed for chemical composition. Ash analysis was done by AOAC method 900.02A. Sand quantification was done using AOAC method 200.02 D. Protein quantification use AOAC method 928.08. Crude fiber and dietary fiber determination use AOAC method 978.10.

C. Extraction method

The crude extract was prepared by dissolve dry *Echinocactus grusonii* powder mg in acetonitrite 10 ml and extract in shaking water bath at temperature of 5, 15, 25, 35, 45˚C for 10, 15, 20, 25, 30 min and centrifuge at 3000 g for 3 minutee. The extraction was repeat three times, The supernatant was filtered and combined adjust volume to be 100 mL then the solvent was evaporate by rotary evaporator. The crude extract was weight for yield percentage and total phenolic quantification[15].

D. Experimental Design

Multiple linear regression was applied to identify optimum levels of three variables of the raw material drying temperature (°C), extraction temperature (°C) and extraction time (min) regarding of two responses yields and total phenolic compounds in the *Echinocactus grusonii* extracts. The design independent and dependent variables are list in Table I. Ranges of drying temperature (X_1), extraction temperature (X_2) and extraction time (X_3) and the central points were selected based on literature review results. The experiments were designed according to the central composite design (CCD) using a 2^3 factorial and star design with six central points as shown in Table I. The order of the experiments has been fully randomized. Data were analyzed by multiple regressions through the least-square method.

Table I Uncoded and coded levels of independent variables used in the experimental design

Symbols	Independent variables	Coded levels				
		− 1.5267	− 1	0	1	1.5267
X_1	Drying temperature	50	60	70	80	90
X_2	Extraction temperature (°C)	15	20	25	30	35
X_3	Extraction time(min)	10	15	20	25	30

III. Results

A. Proximate analysis

The dried *Echinocactus Grusonii* powder composed of ash 20.79 ± 2.90 %, and 19.15 ± 0.14 % , protein 2.03 ± 0.33%, Crude Fiber 17.59 ± 1.29 %, Dietary Fiber 19.10 ± 1.60% and carbohydrate 51.83 ± 3.79 %

B. Extraction condition

The yields of crude extract (Y_1), total phenolic (Y_2) in *Echinocactus grusonii* crude extracts obtained from all the experiments are listed in Table II. The regression coefficients and results of ANOVA show in Table III and IV for response of yield percentage and total phenolic respectively.

IV. Statistical analysis

Data from Table II was analyzed by multiple linear regressions to determine the correlation between independent variable including get optimal extraction condition for highest yield and total phenolic as shown in Table III and Table IV.

TABLE II EXPERIMENTAL DESIGN AND RESPONSES OF THE DEPENDENT VARIABLES TO THE EXTRACT PARAMETERS

Exp. No[a].	Independent variables			Yield	Total phenolic
	Drying temperature (˚C) X_1	Extraction temperature (˚C) X_2	Extraction time (min) X_3	(mg/g dry powder) (Y_1)	(mg GAE/mg. extract) (Y_2)
1	60 (-1)	15 (-1)	15 (-1)	9.00	2.70
2	80 (+1)	15 (-1)	15 (-1)	9.21	2.64
3	60 (-1)	35 (+1)	15 (-1)	9.09	1.51
4	80 (+1)	35 (+1)	15 (-1)	7.76	2.92
5	60 (-1)	15 (-1)	25 (+1)	14.28	6.04
6	80 (+1)	15 (-1)	25 (+1)	7.32	8.30
7	60 (-1)	35 (+1)	25 (+1)	7.00	15.57
8	80 (+1)	35 (+1)	25 (+1)	9.39	10.00
9	70 (0)	25 (0)	20 (0)	5.38	3.20
10	50 [-α]	25 (0)	20 (0)	2.95	6.16
11	90[+α]	25 (0)	20 (0)	2.59	9.31
12	70 (0)	15[-α]	20 (0)	6.19	5.10
13	70 (0)	45[+α]	20 (0)	12.01	3.60
14	70 (0)	25 (0)	10 [-α]	8.60	1.27
15	70 (0)	25 (0)	30[+α]	11.36	9.04

[a] Experiments were conducted in a random order.
[b] mg GAE/g extract (GAE: gallic acid equivalents).
+α= Highest level, + = High level, 0 = Medium level, - = Low level, - α = Lowest level,
$\alpha = 2^{k/4}$ where k is number of variables

TABLE III THE REGRESSION COEFFICIENTS AND RESULTS OF ANOVA FOR YIELD RESPONSE

ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	8.637	3	2.879	0.243[ns]	.864
Residual	130.244	11	11.840		
Total	138.881	14			

ns: no significant at p > 0.05

TABLE IV THE REGRESSION COEFFICIENTS AND RESULTS OF ANOVA FOR RESPONSE OF TOTAL PHENOLIC

ANOVA

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	134.947	3	44.982	5.737*	.013
Residual	86.249	11	7.841		
Total	221.196	14			

* significant different at p > 0.05

From Table 2, highest yield shown at condition of drying temperature at 60˚C, extraction temperature at 15˚C, and extraction time at 25 min ˚C. In addition, when data was analyzed for correlation between independent variable and yield percentage, the statically analysis showed no significant at p value more than 0.05. For optimal extraction condition for highest total phenolic, the drying temperature should be at 60˚C, extraction temperature at 35 ˚C, and extraction for 25 min also correlated with statistically analysis for correlation showed significant at p value less than 0.05.

V. CONCLUSION

The optimal extraction condition to prepare *Echinocactus grusonii* extract with highest yield and total phenolic was determined using CCD experimental design and data was used for statistically analysis by ANOVA. The percentage yield of *Echinocactus grusonii* crude extract is between 2.59 -14.28%. Still, future research for total flavonoids and active glycoside by high performance liquid chromatography should be applied for quantification of active glycoside in *Echinocactus grusonii* and get specific optimal extraction condition

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