IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

DESIGN BASED MICROWAVE ASSISTED EXTRACTION OF CUCUMIS MELO L SUBSP. AGRESTIS (NAUDIN) PANGALO LEAVES AND ITS EVALUATION FOR TOTAL FLAVONOIDS CONTENT

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Abstract:

Cucumis melo L subsp. a*grestis* (Naudin) Pangalo Leaves were extracted using conventional methods generally with low total flavonoids content. In this study *Cucumis melo* L subsp. a*grestis* (Naudin) Pangalo Leaves were extracted by microwave assisted extraction (MAE) and optimization of extraction parameters by using Box Behnken Design on the basis of response factor i.e, total flavonoid content. The selected medicinal plant was extracted microwave assisted extraction as well as other traditional methods (i.e, Soxlet and room temperature). These methods were analyzed on the basis of percentage yield as well as total flavanoid content . To optimize MAE conditions, three main factors were selected using univariate approach experiments and then Box Behnken design (BBD). The optimal extraction conditions were as: Microwave Power (300-700 W); Solvent Concentration (30-70%); Solute and solvent ratio (1:5 - 1:20). Temperature 70^0C, Time 2 min were kept constant. One response factor total flavanoid content (TFC) was used for quantitatively analyze the phytoconstituents in plant part. The optimized conditions for the microwave-assisted extraction of *Cucumis melo* L subsp. a*grestis* (Naudin) Pangalo leaves was microwave power of 562 W, solvent concentration 50% and solvent and drug ratio 20 ml per gm of the drug. The total flavanoid concentration (42.91±0.034) and percentage yield (53) in MAE higher than the other traditional methods(i.e, room temperature, soxlet extraction) with respect to percentage yield(22, 28) and total flavanoid content(22.645±0.021, 32.098±0.104). The BBD can be successfully applied to optimize the extraction parameters (MAE) for *Cucumis melo* L subsp. a*grestis* (Naudin) Pangalo leaf part. Moreover, in terms of environmental impact, the MAE technique could be assumed as a 'Green approach' because the MAE approach for the extraction of plant released only 80.5 gm of CO_2 as compared to 4502.4 gm CO_2 using the soxlet method of extraction. In this work, a fast and efficient microwave assisted extraction (MAE) method was developed to extract bioactive constituents from *Cucumis melo* leaves.

***Index Terms* - *Cucumis melo* L subsp. a*grestis* (Naudin) Pangalo, Leaves, Cucurbitaceae, Total flavonoids content**

I. INTRODUCTION

The Cucurbitaceae family includes most important vegetable crops like cucumber, melon, watermelon, squash, pumpkin etc having worldwide importance. *Cucumis melo* originated in Asia[1,2], and diverse wild and primitive melons are found on that continent, particularly in India [3]. *Cucumis melo* var. agretis Naudin (Panglo) from Cucurbitaceae family also known as kachri, bitter gourd etc. The most probably a varieties of *Cucumis melo* var *agrestis* is *Cucumis callosus* is native to dry areas of India and being common throughout the South America, areas of Thailand, Egypt and Africa, eastward through Iran to India and other parts of tropical Asia[4,5]. Leaves unlobed (or moderately lobed), male flowers in fascicles, roots non-tuberous, annuals. Stem slender, flowers with diameter below 4 cm, pedicel slender, fruits very small below 50 g, often bitter, seeds below 5 mm in length, weedy[6]. *Cucumis melo* subsp. *agrestis* is thought to be the only subspecies of *Cucumis melo* occurring naturally in Australia, with subsp. *melo* being widely cultivated. Kirkbride (1993) recognised subspecies of *C. melo* based on the type of pubescence on the hypanthium of female flowers [7,8].

Microwave assisted extraction has wide acceptance due to shorter time, less solvent consumption, higher extraction rate with better products lower cost and lower decomposing of the target species. It is a powerful tool for sample preparation of solid matrices which combines microwave and traditional solvent extraction [9,10]. The aims of the present work was to evaluate the feasibility of microwave assisted extraction for extraction of total flavanoids. The operational parameters were optimised using single factor experiments and orthogonal experiment design. The objective of the work is to establish the optimised condition of MAE for development and application of the *Cucumis melo* var. agretis Naudin (Panglo) plant.

II. MATERIAL AND METHOD

2.1 Plant material

Cucumis melo L subsp. a*grestis* (Naudin) Pangalo was collected from widely grown region of Southern Haryana in the month of June 2015. The plant was taxonomically identified and authenticated by Dr. Anjula Pandey, Principal Scientist Raw Materials, Herbarium and Museum Division, NISCAIR, New Delhi, vide reference number NHCP/NBPGR/2016-15 *Cucumis melo* var. agretis Naudin (Panglo) dated 17 March, 2016. A voucher specimen of the same has been retained in the Department for the future reference. The *Cucumis melo* var. agretis Naudin (Panglo) leaves and fruits were used to carry out the experimental work.

2.2 Microwave assisted extraction

The plant leaves and fruits were collected separately and dried in shade. The dried pieces of *Cucumis melo* var. agretis Naudin (Panglo) leaves were crushed and finely grind. The finely grinded sample was pass through sieve no 22 and then passes through sieve no 44. This plant sample (1g) was kept in petroleum ether, after 10 minutes, filtered and dried it. Then dried sample was kept in a four necked round bottom flask with solvent ethanol (50%). The sample was extracted in U-Wave 1000 Microwave synthesis reactor (SINEO Microwave Chemistry Technology, China) at Power-time mode [11]. The instrument operates at an input power of 2000W with operating frequency of 2450MHz and works at atmospheric pressure. The real time temperature was monitored by high precision platinum resistance temperature sensor. The flask was connected to outside condenser through a glass connecting tube (19mm U) and a X shaped tube. The pulverized drug was extracted at different operating conditions (Microwave power, Ethanol concentration (%) and different volume of solvent/g of drug) as suggested by experimental design. The extracts obtained by different techniques were cooled for 5 min before filtration. Further the extracts was filtered and concentrated under reduced pressure by a rotary evaporator at 60^0C [12]. The experiment was conducted in triplicate and percentage yield (w/w) was determined. The extracts were kept in a desiccator before further analysis.

2.3 Experimental design

In the present study three process variables like Concentration (X1) and volume of solvent per gram of drug (X2), microwave power (X3) were selected to investigate the effect of these factors on response, i.e. total flavonoids content using Box-Behnken design (BBD). The optimum conditions for the microwave assisted extraction of selected plant were determined with the help of response surface methodology (RSM). As described by design, twenty experiments were conducted representing eight factorial points, six axial points and two central points for validation and suitability of model. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded form is useful for identifying the relative impact of the factors by comparing the factor coefficients (Table 1).

Table:1 Actual and coded values of independent factors

S.No	Parameters	Low level	Middle level	High Level
1	Solvent Concentration (%)	30 (-1)	50 (0)	70 (+1)
2	Solvent per g of drug (ml)	5 (-1)	12.50 (0)	20 (+1)
3	Microwave Power(W)	300 (-1)	500 (0)	700 (+1)

The statistical analysis and significance of proposed model was determined by the application of analysis of variance (ANOVA). The design expert software (11.0, Stat-Ease, Inc. 2021 East Hennepin Ave, Suite 480 Minneapolis, MN 55413) was employed for design, analysis and to draw the response surfaces [13-18].

2.4 Determination of total flavonoids content-

The total flavonoid content of various extracts was determined using a colorimetric method. Briefly, each sample (0.5 ml; 2 mg/ ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a sodium nitrite solution (15% w/v). After 6 minutes, 0.15 ml of an Aluminum chloride solution (10%, w/v) was added and allowed to stand for 6 minutes; then 2 ml of sodium hydroxide solution (4%, w/v) was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank[12,19-21].

Total flavonoid content was calculated using the formula: $FC = Cc \times V/M$

where; FC=total flavonoid content in mg/gm, in QE (Quercetin equivalent); Cq = the concentration of quercetin established from the calibration curve in mg/ml; V=the volume of extract in ml; M=the weight of plant extract in mg. All the tests and analysis were run in triplicates and averaged[22,23].

III. RESULTS AND DISCUSSION

By controlling the extraction time as the only variable factor, the total flavanoid content and yield were detected. The yields had no obvious change. In conclusion, according to multifactorial consideration, we considered that the optimal conditions for MAE total flavanoid content and yield were extracting samples with water, maintaining temperature at 70^0C for 3 min.

Table: 2 Total flavonoids content(R) under various experimental conditions as prescribed by BBD

Run	X_1 Concentration (%)	X_2 Solvent per gm (ml)	X_3 Power (watt)	Yield (%)	R Total Flavanoid Content (mg/gm)	
					Actual	Predicted
1	50.00	20.00	700.00	33	42.91	43.501
2	70.00	12.50	300.00	14	27.944	30.624
3	30.00	5.00	500.00	33	17.668	20.733
4	50.00	12.50	500.00	30	38.324	40.561
5	50.00	20.00	500.00	39	48.703	49.550
6	30.00	12.50	300.00	21	13.158	20.346
7	50.00	12.50	500.00	29	38.986	40.550
8	30.00	12.50	700.00	30	13.779	20.346
9	50.00	12.50	500.00	28	38.91	40.572
10	30.00	20.00	500.00	33	23.468	25.381
11	70.00	12.50	700.00	31	35.269	38.562
12	70.00	5.00	500.00	23	26.034	25.681
13	50.00	12.50	500.00	30	38.503	40.572
14	50.00	12.50	500.00	30	38.565	40.562
15	50.00	5.00	500.00	27	35.482	36.620
16	50.00	5.00	300.00	30	24.115	25.631
17	50.00	5.00	700.00	18	28.862	30.583
18	50.00	20.00	300.00	30	33.124	35.572
19	70.00	20.00	500.00	39	39.441	40.879
20	50.00	12.50	500.00	30	38.802	40.560

3.1 Comparison of MAE with other methods

For comparison with MAE, we investigated conventional Soxlet extraction and Room temperature (RT) extraction. Total flavanoid content and percentage yield were measured. MAE was the most efficient among the three methods. Total flavanoid content and yield with MAE were at least 2 times higher than those with Soxlet extraction and RT extraction. The Soxlet extraction method had the same conditions as MAE, except for microwave radiation and ethanol water concentration[24].

Table 3: ANOVA for Quadratic model, Response: TFC

Source	Sum of Squares	Mean Square	F-value	p-value
Model	13.96	1.55	17.25	< 0.0001
A-Concentration	3.60	3.60	40.04	< 0.0001
B-Solute/ Solvent	1.12	1.12	12.41	0.0055
C-Power	0.4970	0.4970	5.53	0.0406
AB	0.0252	0.0252	0.2802	0.6081
AC	0.0807	0.0807	0.8972	0.3659
BC	0.0278	0.0278	0.3095	0.5902
A^2	6.20	6.20	69.00	< 0.0001
B^2	0.1152	0.1152	1.28	0.2840
C^2	0.7746	0.7746	8.62	0.0149
Residual	0.8990	0.0899		
Pure Error	0.0024	0.0003		
Cor Total	14.86			
Std. Dev.	0.2998			
R^2	0.9395			
Adjusted R^2	0.8850			
Adeq Precision	12.1823			

The model is significant. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 12.182 indicates an adequate signal. This model can be used to navigate the design space. The Model F-value of 17.25 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, A^2, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1[25-28]. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$TFC = 6.22+0.6708A+0.3734B+0.2492C-0.0794AB+0.1420AC+0.0834BC-1.16A^2-0.1588B^2-0.4116C^2$$

3.2 Diagnostics of model adequacy

The adequacy of developed model was further evaluated by diagnostic plots. The normal plot of residual between normal % probability and internally studentized residuals was normally distributed with no significant deviation of variance which justifies the fitness of developed model (Table 3). The plot between predicted and actual response clearly indicated that all the predicted values lie near to the straight line and was in agreement with real values (Figure 1).

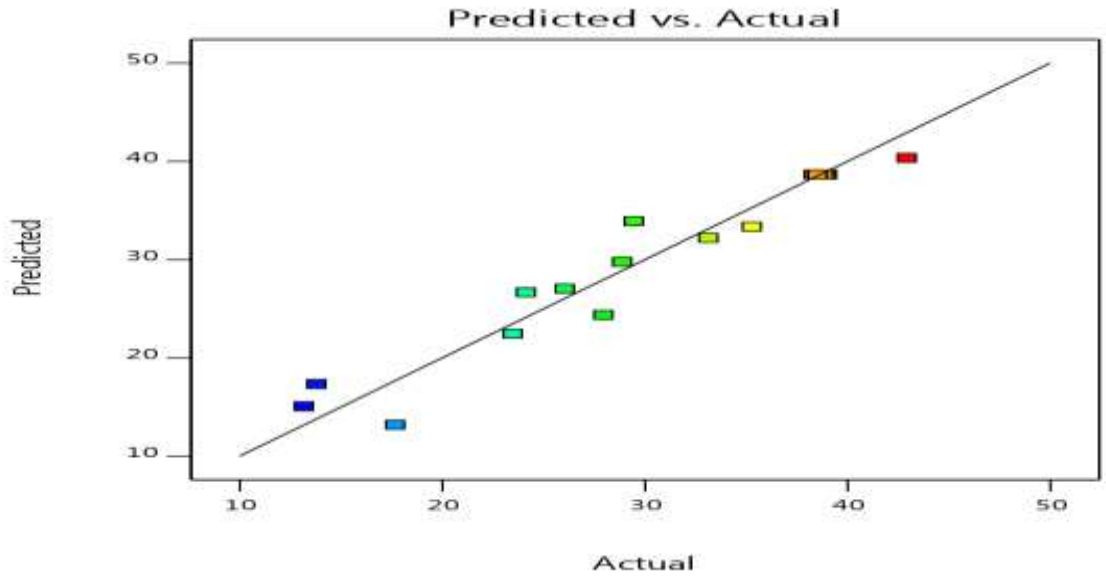

Figure 1: Plot between predicted and actual response

3.3 Response surface analysis

The 3D response surface graph predicts the relationship between the response and two process variables, keeping the third factor at zero level. The effect of microwave power (X_3) and concentration (X_1) on total flavanoid content (R) was shown in Figure 6(a), while the solvent/drug ratio (X_2) was kept at zero level. It was clear from the figure that the total flavanoid content increases linearly with power up to 500 W, after that rise in response was not so significant up to 700W and further increases in power slightly decrease the total flavonoids content (mg/gm), because higher power would deteriorate the chemical structure of active compound to cause decrease in its yield. Since the total flavonoids content increased linearly with concentration up to 50 %, after this concentration decrease in the total flavonoids content. It could be explained as the power rises with concentration more it would more dissipated as heat inside the plant cell and extract. Moreover, the solvent molecules also get dissociated into charged particles (ions) and their flow inside the vessel was also enhanced under the effect of applied electric field. From the response surface plot of concentration (X_1) and solvent to drug ratio (X_2) at a fixed power level (500 W), it was observed that the concentration of total flavonoid content increased steadily up to 50% concentration in Figure 6(b), but solvent to sample ratio have significant impact on the total flavonoids content, increases linearly. Further, the large solvent to feed ratio results in slight increase in the total flavonoids content from the extract [11,18, 28-30]. This observation was possibly due to fact that the large volume of solvent requires the additional power for effective heating which in turn disturbs the chemical nature of constituents. The 3D plot between the power (X_3) and solvent per g of drug (X_2) for 50% concentration established the relationship independent variable (X_1) and dependent variable (R) in Figure 6(c). The observation of plot reveals the linear rise of total flavonoids with solvent concentration up to 20 ml/g of drug, because the optimum amount of solvent breaks the mass transfer barrier and promotes the movement of plant actives out of the cell matrix [31].

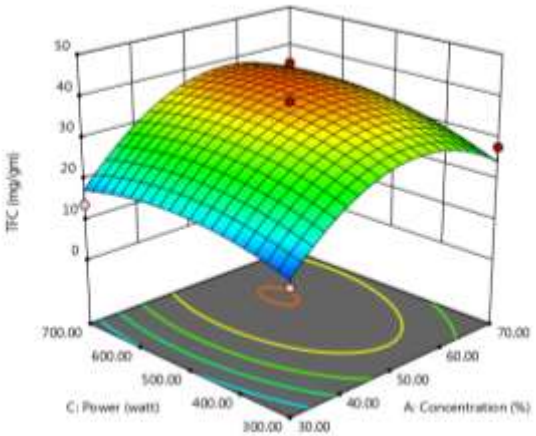

Figure 6(a)

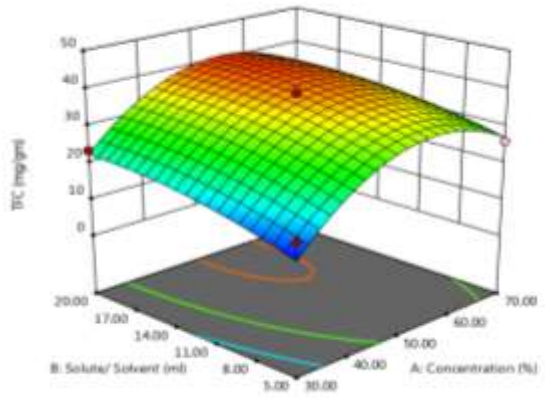

Figure 6(b)

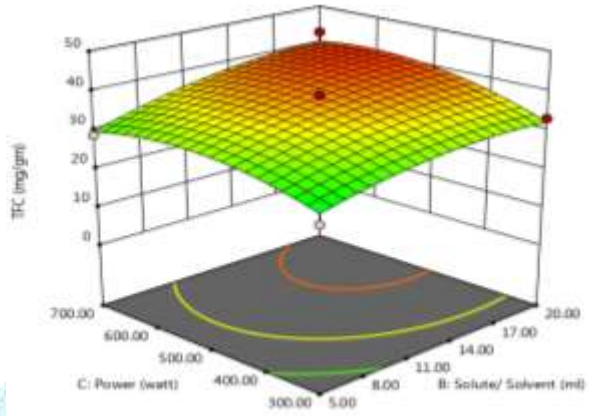

Figure 6(C)

3.4 Optimization of Extraction Conditions

The process variables, viz. solvent concentration, solvent to sample ratio, and power were numerically optimized to find out the conditions for getting the best results from MAE extraction. Optimization was done for obtaining maximum total flavonoids content. The optimal conditions of MAE for total flavonoids content extract obtained were found as solvent concentration of 50 %, solvent to sample ratio of 20:1, and power of 532 watt. For the validation and adequacy of the model equation, a verification experiment was carried out under the optimized conditions mentioned above. The predicted R^2 total flavonoids content of extract in quadratic source was 0.875, which was consistent with the practical total flavonoids content of 0.8912 of extract and thus validates the model[28]. The result given in the table 3 shown the linear, quadratic and interaction effects of the three factors on the responses. Linear and quadratic term of concentration showed the significant effect on TFC.

3.5 Comparison of MAE with Conventional Extraction Methods

The efficacy of MAE was compared with those of conventional extraction methods (Soxlet extraction and Room temperature), and the results are displayed in Table 4. The MAE process improved the yield compared with soxlet as well as room temperature extraction. Furthermore, the MAE process significantly reduced the extraction time than the room temperature method (3 min vs. 24 h). In comparison with Soxlet extraction, the MAE process significantly increased the extraction efficacy by 44%, and meanwhile required lower temperature (70 C vs. 80 C) and shorter time (3 min vs. 8 h). The underlying mechanism of MAE is that microwave energy could cause molecular motion via ionic conduction and dipole rotation. Additionally, the extract obtained by MAE method showed higher total flavonoid content (TFC) than the other two extraction methods, which further demonstrated the high efficiency of MAE[30].

Table: 4 Comparison of MAE with conventional methods

S.No.	**Extraction Methods**	**Ethanol Concentration (%)**	**Temperature (C)**	**Time**	**Yield of extract (%)**	**TFC(mg QE/g DW)**
1	Room temperature	70	28	24 h	22	22.645±0.021
2	Soxlet	70	80	8h	28	32.098±0.104
3	MAE	70	70	3 min	53	42.91±0.034

3.6 Green Audit

In MAE optimal conditions (at 500W and for 180 s) significantly reduced the time as well energy as compared to Soxlet Extraction (at 100W for 8 h). The energy consumption was decreases from 0.8 KWH in conventional method to 0.025 KWH by MAE process. The novel technique of extraction (MAE) could be assumed as 'Green approach' for extraction of *Cucumis melo* var. Agretis Naudin (Panglo) leave part. As this process release only 80.42 gm of CO_2 relative further, in the present study the solvent ratio per g of the drug was also optimized to 20:1 which suggest the less consumption of solvent in MAE process in comparison to the Soxlet method of extraction (50:1). The comparative data of various extracts is summarized in Table 3.

3.7 Conclusion

The present research work concludes that the central composite design coupled with response surface methodology can be successfully applied to optimize the extraction parameters (MAE) for *Cucumis melo* var. agretis Naudin (Panglo). The study reveals that extraction of selected medicinal plant by microwave based approach at optimized conditions almost doubles the extract yield and total flavanoid concentration with less consumption of solvent and electricity as compared to traditional method. Moreover, the total flavonoids content of the MAE extract enhaKnced significantly relative to the SME. The enhanced total flavonoids concentration may also be due to altered concentration of phenolic or flavonoid compounds which can be further evaluated. The results were also supported by microscopic analysis of drug samples. To conclude, it can be said that MAE technique for extraction of whole plant of *Cucumis melo* var. agretis Naudin (Panglo) could be commercially utilized for scale-up process. Further MAE could be assumed as green approach for the extraction with high returns on capital investment. Finally the research could be further extended to analysis of extracts for other Phytoconstituents and also the variability of contents with different collection time of raw material.

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