Research Article

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QUANTITATIVE ESTIMATION OF CUCURBITACIN E IN VARIOUS EXTRACTS OF *CUCUMIS SATIVUS* L. BY SPECTROPHOTOMETRIC METHOD

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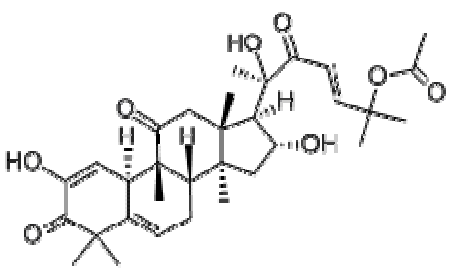
ABSTRACT

Cucumis sativus L. is a annual climber belongs to the family Cucurbitaceae. Cucumber is a native to the tropics and is one of the oldest cultivated vegetable crop .*Cucumis sativus* is growing widely throughout the Bangladesh, Indian subcontinent, Sri Lanka .It is an important medicinal plant with diverse pharmacological spectrum widely used in Ayurveda, Siddha, Chinese medicine etc .Plant has many important phytoconstituents like glycosides, flavones, terpenoids, phytosterol, saponins etc responsible for many of the pharmacological activities such as antibacterial, antifungal, antidiabetic, cytotoxic, antacid, hepatoprotective activity, wound healing activities .The aim of the present study was to develop and validate an analytical method to determine the Cucurbitacin E content in extract from *Cucumis sativus* leaves, stems and seeds, by direct and PMA reaction UV/Vis spectrophotometric method. The crude extract was used to develop a method for Cucurbitacin E assay .The optimum conditions for wavelength and analysis time were 263.50 nm; and 10 min, 400.40 nm for direct UV method and PMA reaction method respectively .Under these conditions, validation as per ICH guidelines proved the method to be linear, specific, precise, accurate, reproducible, robust, and easy to perform .This methodology complies with the requirements for analytical application and to ensure the reliability of the results.

KEYWORDS: *Cucumis sativus* L., Cucurbitacin E, PMA reaction method, ICH guidelines.

INTRODUCTION

Cucurbitacins are a group of highly oxygenated tetracyclic triterpenes contains cucurbitane skeleton well known for bitterness and toxicity, are of great interest because of the wide range of biological activities they exhibit in plants and animals. They are predominantly found in the Cucurbitaceae family. A relatively common cucurbitacin found in Cucurbitaceous species is cucurbitacin E (CuE). Cucurbitacin E (CuE, α-elaterin) is an active compound, previously shown to be a strong antifeedant with the ability to disrupt cellular actin and cell adhesion. CuE has an inhibitory effect on cancer cell proliferation, actin polymerization, and permeability. Also number of compounds of this group has been investigated for their cytotoxic, hepatoprotective, cardiovascular, antidiabetic effects, antioxidant effects of cucurbitacins B and I and the glucosides of cucurbitacin I and L. Cucurbitacin B was also shown to exhibit anti-inflammatory activity. Additionally, several studies indicated that different cucurbitacin species inhibit the proliferation of cancer cells through different mechanisms.[1-7]



Cucurbitacin E

Cucumis sativus Linn. (Family: Cucurbitaceae) is an annual, rather coarse, fleshy, prostrate or climbing vine. It is widely distributed all over the world particularly in Asia, Africa and South America. Traditionally, this plant is used for headaches; the seeds used as cooling and diuretic, the fruit juice is used as a nutritive and as a demulcent in anti-acne lotions. Several investigations revealed antidiabetic, antiulcer, moisturizing, antioxidant and analgesic property of the fruit extracts. The seed extracts were found effective on controlling the loss of body weight in diabetic rats and against tapeworms. Cytotoxic, antifungal and antibacterial activity activities have been reported from leaves and stems extracts. [8-15]

Colorimetric reactions are widely used in the UV/Vis spectrophotometric method, which is easy to perform, rapid and applicable in routine laboratory use and low cost. Phosphomolybdic acid is widely employed as a reagent in quantitative analysis of several drugs. It forms insoluble adduct with various groups of drugs and released PMA from adduct is usually measured by colorimetrically due to blue-green color formation by reduction of PMA. Also color reactions do not require stringent conditions nor many reagents or solvents. Advantage of the method is that there is neither extraction procedure nor interference of other ingredients. Also results achieved in shorter time but still with great consistency.[16]

Development of quantitative method for cucurbitacin analysis can contribute to the standardization and quality control of drug as well as its extracts. This control is important to guarantee safety and efficacy in the use of any pharmaceutical products, including medicinal plants. Due to toxicity and wide variety of pharmacological activities it becomes necessary to determine the content of the cucurbitacin. In this regard we have developed

and validate UV method for quantification of the major constituent Cucurbitacin E from leaves, stems and seeds parts of *Cucumis sativus* by direct UV method and PMA reaction method.

MATERIAL AND METHODS

Standards and Chemicals

All chemicals were analytical-reagent grade and the water was distilled. The chemicals included Phosphomolybdic acid reagent, Cucurbitacin E (Sigma-Aldrich), DMSO.

Plant Material

Fresh and fully grown plant of *Cucumis sativus* was collected from fields of Khanna, Punjab (India) in month of May and June. Plant were subjected to first the morphological identification as described in the literature and followed by authentication by Dr. Sunita Garg, Chief Scientist and Head, Raw Materials Herbarium and Museum (RHMF), NISCAIR, New Delhi. The herbarium voucher specimen of plant preserved in the department of Chandigarh College of Pharmacy, Landran (Mohali) for future reference CCP/HB/RK/06.

Extraction

The plant material (Leaves, stems and seeds) was washed with water to remove soil, dried in air under shade at room temperature (25± 2 ^{o}C) and reduced to coarse powder in a mixer grinder. Using Soxhlet apparatus successive extraction was carried out with methanol after defatting with petroleum ether. The liquid extracts obtained were concentrated and dried in vacuum desiccators and weighed and stored at -20 °C.

Method Optimization and Standardization for Determination of Cucurbitacin E Content

Direct UV Method.

Optimization and standardization of cucurbitacin E content by direct UV spectrophotometric method were analyzed by maximum absorption wavelength method. For this reference standard, Stock solution (Cucurbitacin E,10µg/ml) and all the extract of leaves, stems and seeds, were prepared in DMSO and diluted with distilled water and were scanned in UV visible spectrophotometer (Shimazdu-1800) from 200-800nm to determine the spectra using distilled water as blank with quartz cell (1cm path length). The wavelength selected was 263.50nm for development of UV method for estimation of CuE in methanolic extracts of leaves, stems and seeds of *Cucumis sativus*.

PMA Reaction Method

For the optimization and standardization of the spectrophotometric method using the PMA reagent, two parameters were analyzed: (1) reaction kinetics, (2) maximum absorption wavelength. Reaction method involve reacting all samples (standard Cu E and ME extracts solution) with 2% phosphomolybdic acid (PMA) solution in absolute ETOH at room temperature in 1:1 ratio. Stock solution of standard and extracts of leaves, stems and seeds were prepared in DMSO and diluted with water. 2ml of PMA was added to the solution and allowed to stand for 5, 10, 15 and 20 minutes. Then samples were scanned with UV spectrophotometer (Shimazdu-1800) from 200 to 800nm, to determine spectra and compared the absorbance difference with respect to time. Distilled water was used as blank with the quartz cell (1cm path length). The wavelength selected was 400.40nm for development of reaction method for estimation of CuE in methanolic extracts of leaves, stems and seeds of *Cucumis sativus*. The kinetics reaction was evaluated by comparing the percentage increase in absorbance of each solution for the wavelengths 390, 395, 400.4, 405 and 410nm. This percentage increase was calculated by dividing the difference in absorbance between two wavelengths by the mean absorbance of the shorter wavelength and multiplying by 100. The reaction times were observed and compared statistically, using the percentage increase between reaction times (5, 10, 15 and 15 minutes) at 400.40nm. The specific absorptivity of standard was calculated based on Lambert-beer's law.[16]

Preparation of Calibration curve by Direct UV method

Standard stock solution of Cucurbitacin E was prepared by dissolving accurately weighed 1mg of CuE in DMSO in a 10ml volumetric flask which gives concentration of 100µg/ml. From this stock solution, 1ml solution was pipette out into 10ml volumetric flask and finally made up the volume with water to produce a concentration of 10µg/ml. From the stock solution similarly further dilutions (10-100µg/ml) were made. Then absorbance of all the solutions was determined at 263.50nm.

Accurately weighed (10mg) amount of extracts (leaves, stems and seeds) was dissolved in DMSO in 100ml of volumetric flask (100µg/ml) and for further dilutions (10-100µg/ml) were prepared from this stock solution. Absorbance was measured at 263.50nm to determine CuE content in the extract solutions.

Preparation of Calibration curve by PMA Reaction Method

Standard stock solution of Cucurbitacin E was prepared by dissolving accurately weighed 1mg of CuE in DMSO in a 10ml volumetric flask which produce concentration of 100µg/ml and further dilutions (1-10µg/ml) was prepared from this stock solution. All the solutions were subjected to further reaction with 2ml phosphomolybdic acid solution for 10 minutes and finally diluted to 10ml with water then the absorbance was measured at 400.40nm.

Accurately weighed (10mg) amount of extracts (leaves, stems and seeds) was dissolved in DMSO in 10ml of volumetric flask (100µg/ml) and for further dilutions (1-10µg/ml) were prepared from this stock solution. All dilutions were subjected to react with 2ml of PMA and allowed to stand for 10 minutes. Then absorbance was measured at 400.4nm to determine CuE content in the extract solutions.

Analytical Method Validation

For validation of the analytical method, the guidelines established by the ICH (International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use) were employed.[17-19]

Linearity

To establish the linearity of proposed methods, various aliquots of standard and extracts (leaves, stems and seeds) were prepared from respective stock solution (100µg/ml) for direct UV and (100µg/ml) for PMA reaction method in three replicates and absorbance was measured. Linearity curve was obtained by plotting concentration vs. absorbance. From the curve linear range was determined.[17-19]

Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. It is determined by adding 1 ml of standard solution (40µg/ml) direct UV and (0.6µg/ml) for PMA reaction method to each stock solution described in the linearity test. The method is considered specific if the slope of linear equation in the tests for linearity and specificity are equal and very similar.[18]

Precision

Precision was carried out to ascertain the reproducibility of proposed methods. Repeatability was determined by preparing a six replicates of the sample and absorbance was measured. Intraday precision was carried out by preparing solution of same concentration and analyzing at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as % RSD. The method is considered precise if % RSD is ±2%.[18]

Accuracy

Accuracy of the proposed methods was determined by recovery method. The solutions were prepared in triplicates for direct UV method and PMA reaction method and the known amount of standard solution is added at three concentration level (lowest, medium and highest; 80%, 100%, &120%). The accuracy was assessed as the % recovery which can be calculated from equation:

$$\% \text{ recovery} = (A/A_T)*100$$

Where, A= Absorbance of sample after addition of standard, $A_{T=}$ Theoretical absorbance calculated for the sum of absorbance of extracts and the expected absorbance of standard based on the calibration curve for each level.

This method is considered accurate if the recovery % is between 85% - 115%.[17]

Robustness and Ruggedness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. Robustness in direct UV method dilutions were prepared at different wavelength (262.5 and 264.5nm) and in PMA reaction method different quantities (1ml and 3ml) of PMA were added and the respective absorbance was noted and the result was evaluated in three replicates and expressed as% RSD. [18]

LOD and LOQ

The LOD and LOQ were calculated from the relationship between standard deviation (SD) of the crude extract linearity and slope using the appropriate multiplier. These results were compared with LOD and LOQ obtained by extension of linearity curve of crude extracts of *Cucumis sativus.* The results, together with the results for linearity were submitted to statistical analysis. The quantification range was detected based on the lowest and highest concentration that maintained linearity. The LOD was detected as the lowest concentration that was significantly different from the next lowest concentration. [18]

Statistical Analysis

Data were expressed as mean ± standard deviation, relative standard deviation (%). The linear correlation tests and residual analysis were performed by simple linear regression, considering r^2 equal to or higher than 0.99 evaluated.

Table 1: Statistical data of regression equations of calibration curve for CuE& linearity and specificity test for CuE Content from ME of *Cucumis sativus* by Direct UV method

Regression Analysis		Linearity of (Direct UV method)	Specificity of (Direct UV method)
Regression equation	standard CuE	y=0.0047x-0.0326	
	Leaves	y=0.0025x+0.0024	y=0.0025x+0.0126
	Stems	y=0.0028x+0.0036	y=0.0028x+0.015
	Seeds	y=0.0031x+0.0051	y=0.0031x+0.0137
Slope	standard CuE	0.0047	
	Leaves	0.0025	0.0025
	Stems	0.0028	0.0028
	Seeds	0.0031	0.0031
Intercept	standard CuE	0 .0326	
	Leaves	0.0024	0.0126
	Stems	0.0036	0.015
	Seeds	0.0051	0.0137
Regression coefficient	standard CuE	0.999	
	Leaves	0.998	0.997
	Stems	0.998	0.998
	Seeds	0.998	0.998
Linear Range(µg/ml)	standard CuE	10-90	
	Leaves	05-50	
	Stems	10-60	
	Seeds	01-50	

Table 2: Interday and intraday Precision for Direct UV method

(CuE) Conc. (40µg/ml)	Interday Precision Absorbance			Intraday Precision Absorbance		
	Leaves	Stems	Seeds	Leaves	Stems	Seeds
Mean*	0.102	0.115	0.129	0.101	0.115	0.129
SD*	0.001	0.001	0.002	0.001	0.002	0.002
%RSD	0.980	0.869	1.550	0.990	1.739	1.550

(*) Intraday Precision n=6 Interday Precision n=3

Table 3: Accuracy of CuE content by Recovery Method in ME by Direct UV method

Level	% Recovery		
	Stems	leaves	Seeds
80%	96.07	96.55	94.44
100%	100.15	97.14	95.58
120%	103.03	102.70	95.45

*The data is the result of triplicate analysis

Table 4: Method Validation Parameters Direct UV method

Parameters	Leaves	Stems	Seeds
Detection wavelength (nm)	263.50	263.50	263.50
Linearity range (µg/ml)	5-50	10-60	1-60
Slope	0.0039	0.0036	0.0038
Intercept	0.0024	0.0036	0.0051
Regression Equation	y=0.0025x+0.0024	y=0.0028x+0.0036	y=0.0031x+0.0051
Regression coefficient (r2)	0.998	0.998	0.998
Limit of Detection (LOD) µg/ml	1.84	1.17	0.363
Limit of Quantitation (LOQ) µg/ml	5.6	3.571	1.065

Table 5: Statistical data of regression equations of calibration curve for Cu E & linearity and specificity test for CuE content from ME of *Cucumis sativus by* PMA Reaction method

Regression Analysis		Linearity (PMA Reaction method)	Specificity (PMA Reaction method)
Regression equation	Standard CuE	0.18x +0.044	
	Leaves	0.155x+0.029	0.152x+0.036
	Stems	0.155x+0.025	0.154x+0.032
	Seeds	0.123x+0.033	0.124x+0.038
Slope	Standard CuE	0.18	
	Leaves	0.155	0.152
	Stems	0.155	0.154
	Seeds	0.123	0.124
Intercept	Standard CuE	0.044	
	Leaves	0.029	0.036
	Stems	0.025	0.032
	Seeds	0.033	0.038
Regression coefficient (r^2)	Standard CuE	0.999	
	Leaves	0.998	0.997
	Stems	0.998	0.997
	Seeds	0.999	0.999

Table 6: Intraday and Interday Precision for PMA reaction method

(CuE) Conc. (0.6µg/ml)	Intraday Precision Absorbance			Interday Precision Absorbance		
	Leaves	Stems	Seeds	Leaves	Stems	Seeds
Mean	0.117	0.115	0.106	0.119	0.116	0.107
SD	0.002	0.001	0.001	0.001	0.001	0.001
%RSD	1.709	0.869	0.943	0.840	0.862	0.934

Table 7: Accuracy of CuE content by Recovery Method in ME by PMA Reaction Method

Concentration Levels	% Recovery*		
	Leaves	Stems	Seeds
80%	94.68	95.34	97.91
100%	98.00	93.47	99.00
120%	98.11	100	97.16

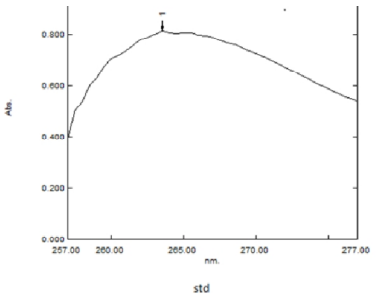
*The data is the result of triplicate analysis.

Table 8: Method Validation Parameters

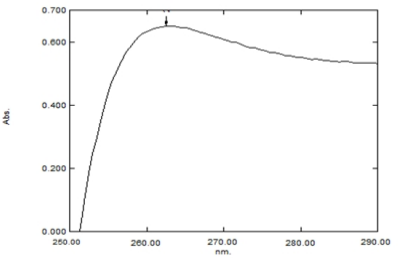
Parameters	Leaves	Stems	Seeds
Detection wavelength (nm)	400.40	400.40	400.40
Linearity range (µg/ml)	0.1-0.6	0.1-0.6	0.1-0.7
Slope	0.155	0.155	0.123
Intercept	0.029	0.025	0.034
Correlation coefficient	y=0.155x+ 0.0293	y=0.155x+0.0252	y=0.123+0.0336
Regression coefficient (r2)	0.998	0.998	0.999
Limit of Detection (LOD) µg/ml	0.021	0.022	0.026
Limit of Quantitation (LOQ) µg/ml	0.065	0.064	0.081

Table 9: Comparison of CuE content by Direct UV and PMA reaction method

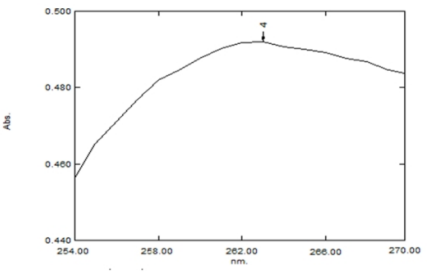
Plant Part	Cu E content by PMA Reaction Method (%w/w)	Cu E content by Direct UV Method (%w/w)
Leaves	4.414	4.703
Stems	3.271	4.061
Seeds	8.713	13.686



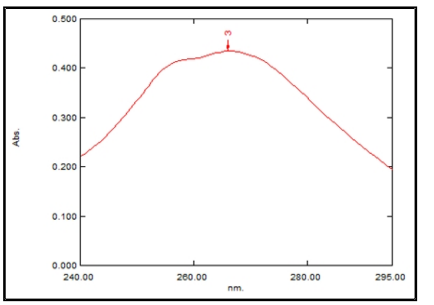
(a)Standard Cucurbitacin E



(b)ME of Leaves



(c)ME of Stems



(d)ME of Seeds

Figure 1(a-d): UV spectrum of Standard Cucurbitacin, ME extract of leaves, stems and seeds part of *Cucumis sativus* with λ_{max} at 263.50nm

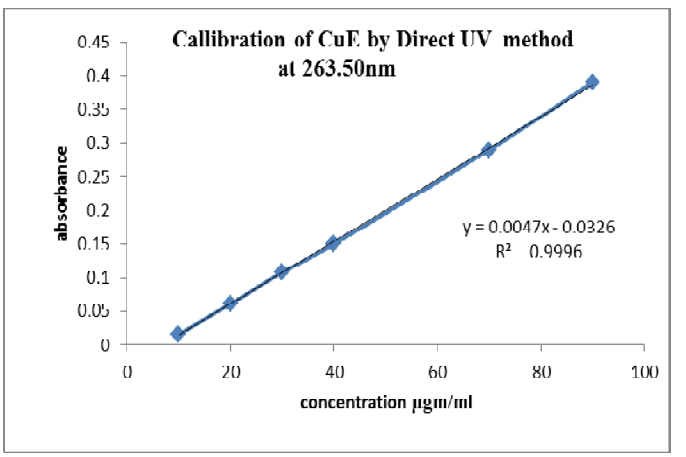
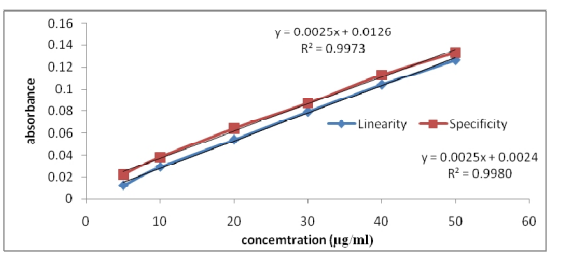
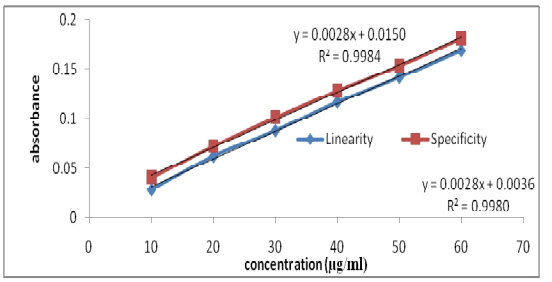


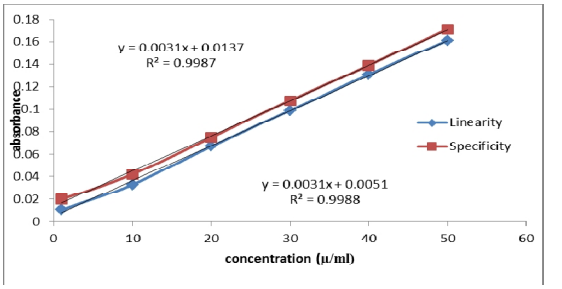
Figure 2: Calibration curve of CuE by Direct UV Method at 263.50nm



(a)Leaves

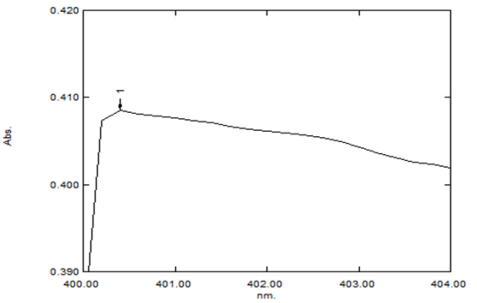


(b) Stems

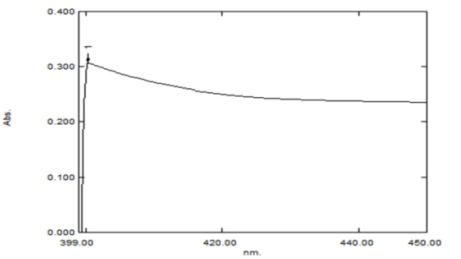


(c)Seeds

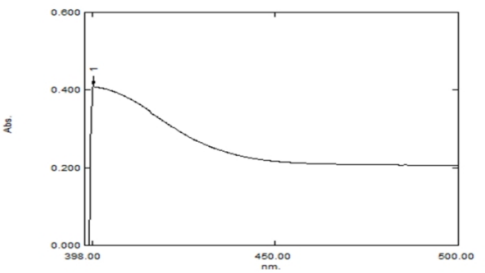
Figure 3(a-c): Linearity curve & specificity curve, with correlation coefficient (r^2) and linear equation for TP in ME from *Cucumis sativus* by Direct UV method



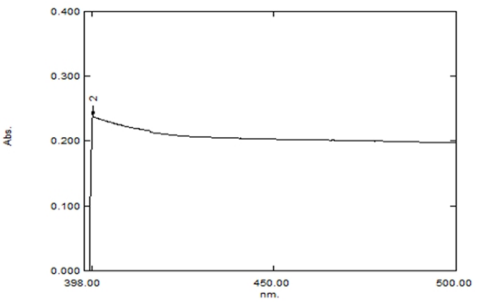
(a)Standard Cucurbitacin E



(b) ME of Leaves



(c) ME of Stems



(d) ME of Seeds

Figure 4(a-d): UV spectrum of Standard Cucurbitacin E, ME of Leaves, Stems and Seeds part of *Cucumis sativus* after PMA reaction with λ_{max} 400.40nm

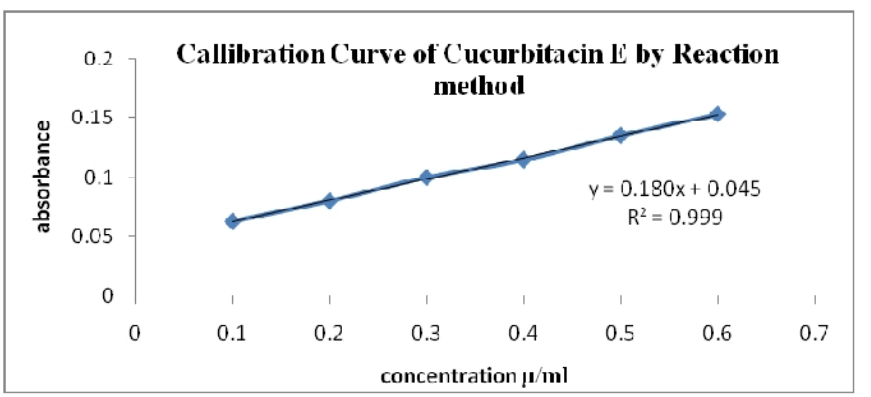
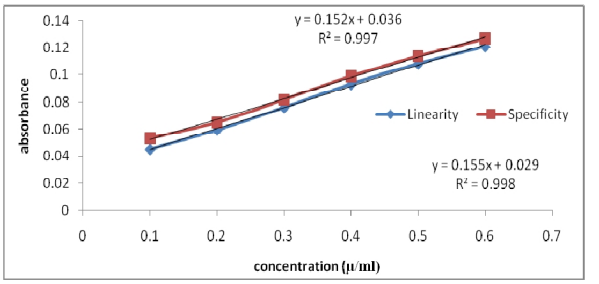
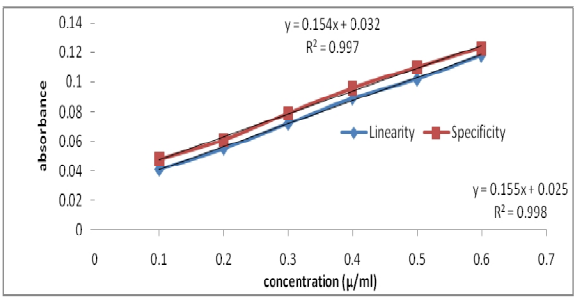


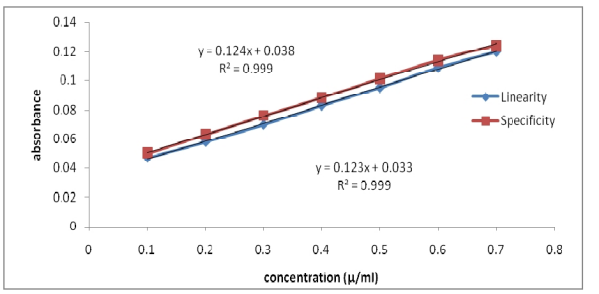
Figure 5: Calibration curve of CuE after PMA reaction at 400.40nm



(a)Leaves



(b)Stems



(c) Seeds

Figure 6(a-c):Linearity curve & specificity curve, with correlation coefficient (r^2) and linear equation for CuE content in ME from *Cucumis sativus* by PMA Reaction method

RESULTS AND DISCUSSION

Method Optimization

Each plant has a characteristic chemical composition with uniform triterpenoids groups present in the same species. Similar chemical structure may show the same chemical interactions with specific reagents during the reactions. Both reference substances and extracts from *Cucumis sativus* show approximately same spectra by direct UV method and by reaction with phosphomolybdic acid reagent.

Direct UV Method

The percentage increase in absorbance of each solution was compared at wavelength of 255, 263, 268 and 270nm. The statistical analysis indicated a significant increase in absorbance at 263.50nm compared to other wavelengths for all solutions. So 263.50nm appeared to be best suited to produce maximum absorption as the methanolic extract (ME) of leaves, stems and seeds from *Cucumis sativus* also show approximately same spectra. (Figure 1(a-d))

Calibration curve were prepared which was found to be linear with $r^2 = 0.999$ and linear regression equation was found to be Y=0.0047x-0.0326 (n=6) for CuE. The relative standard deviation (RSD's) of slopes were ≤ 5% for the analyte (n=6) (Figure 2). The back-fit calculations using calibration curve data for standard were used in the validation runs as well as the precision and accuracy tests.

Method Validation

Linearity

Based on linear regression analysis the responses for the ME in related concentrations ranges were linear. The calibration curve of ME of *Cucumis sativus* was found to be linear with excellent correlation coefficient $r^2 = 0.999$.The typical calibration curve of ME have the regression equation Y=0.0025x+0.002 for leaves; Y=0.0028x+0.003 for stems; Y=0.003x+0.005 for seeds. The Linearity range for leaves, stems and seeds was found to be 5-50µg/ml, 10-60µg/ml and 1-50 µg/ml respectively.

Specificity

Analysis of the results of the specificity test indicated that the conditions were satisfactory. In the case of complex matrices, if the matrix without the analyte is not available, the effects of matrix system can be tested by comparing the slopes of linearity and specificity. If the curves are parallel, we can state that the method is specific linearity and specificity.

The specificity of the method for ME was confirmed by super imposing the analytical curves (Figure 3(a-c)), because the slopes of the linear equations (linearity and specificity, Table 1) were very similar.

Precision

The repeatability and the intermediate precision for ME were with no significant difference between them therefore, the proposed method is adequately precise within the prescribed limits of ICH guidelines (%RSD < 2) (Table 2).

Accuracy

The result for the accuracy test showed percentage recovery to be in range of 96.55% -102.7%, 96.07% - 103.03% and 94.44-95.58%, for leaves, stems and seeds respectively (Table 3). These percentages were within the range of 85%-115% established in published reports. This indicates that the method has good accuracy for determining CuE content in ME from *Cucumis sativus*.

Robustness and Ruggedness

The robustness was demonstrated by analyzing the stability of the solution under the influence of change in wavelength (262.5nm and 264.5nm), evaluated in three replicates. The values for absorbance measure after changing the wavelength and compared with results at 263.50nm the % RSD was found to be less than 2% (%RSD).

Ruggedness shows no significant difference between the analysis carried by two analyst using same experimental conditions and %RSD by both analyst was less than 2.

Limits of Detection and Quantification
Based on the linear equation from the test for linearity, the LOD was found to be 1.84 µg/ml, 1.17µg/ml and 0.363 µg/ml and LOQ was found to be 5.6 µg/ml, 3.57 µg/ml and 1.065 µg/ml for extracts of leaves, seeds and stems respectively (Table 4).

PMA Reaction Method
Phosphomolybdic acid (PMA) reaction method were optimized and standardized for two parameters (i) maximum absorption wavelength, (ii) reaction kinetics. The percentage increase in absorbance of each solution was compared at wavelength of 390, 395, 400.4 and 410 nm. The statistical analysis indicated a significant increase in absorbance at 400.40 nm compared to other wavelengths for all solutions. So 400.40 nm appeared to be best suited to produce maximum absorption of substance under study, the next step was to determine the reaction kinetics with respect to time period prior to spectrophotometric measurement. The increase in percentage absorbance of each solution relative to time period of 5, 10, 15 and 20 minutes were determined. The data showed that the reaction was stable during the period analyzed since after 10 min the absorbance was increased less than 5 % of the value at 5 min and did not decrease between 10 and 15 min. The method was optimized for extracts of *Cucumis sativus* and similar results were obtained.

The data showed as the methanolic extract (ME) of leaves, stems and seeds from *Cucumis sativus* also show approximately same spectra. Figure 4(a-d) Spectra revealed that 400.40nm to be best suited wavelength to produce a maximum absorption. Form standard solution, series of dilutions (0.1-10µg/ml) of extracts were prepared and 2ml of PMA solution was added and then absorbance was measured at 400.40nm.Calibration curve was prepared which was found to be linear with r^2= 0.999 and linear regression equation Y=0.18x+0.044 for CuE. The relative standard deviation (RSD's) of slopes were ≤ 5% for the analyte (n=6) (Figure 5). The back-fit calculations using calibration curve data for standard were used in the validation runs as well as the precision and accuracy tests.

Method Validation
Linearity
The calibration curve for ME were found to be linear with correlation coefficient r^2=0.998. The typical calibration curve of ME have the regression equation y=0.155x+0.0293 for leaves, y=0.155x+0.0252 for stems and y=0.123x+0.0336 for seeds. Linearity range for ME of leaves, stems and seeds of *Cucumis sativus* was found to be 0.1-0.6 µg/ml,0.1-0.6 µg/ml,0.1-0.7 µg/ml respectively.

Specificity
Analysis of the results of the specificity test indicated that the conditions were satisfactory. The effects of the matrix system can be tested by comparing the slopes of linearity and specificity. The specificity of the method for ME was confirmed by superimposing the analytical curves (Figure 6(a-c)), because the slopes of the linear equations (linearity and specificity, Table 5) were very similar**.**

Precision
The repeatability was assessed using six samples of 0.6µg/ml of ME, and the intermediate precision was assessed using three samples and on three different days. The repeatability and the intermediate precision for ME were found with no significant difference between them therefore, the proposed method is adequately precise within the prescribed limits of ICH guidelines (%RSD < 2). (Table 6)

Accuracy
The result for the accuracy test showed a percentage recovery was in range of 94.68-98.11%, 95.34-100% and 97.16-99% for leaves, stems and seeds respectively (Table 7). These percentages were within the range of 85%–115% established in published reports. This indicates that the method has good accuracy for determining Cu E content in ME from *Cucumis sativus*.

Robustness and Ruggedness
The robustness was demonstrated by analyzing the stability of solution by addition of PMA (2ml and 3ml) evaluated in three replicates. The values for absorbance measured after adding the PMA in the reaction method were with no significant difference for leaves, stems and seeds extracts. However, there was a tendency for the proposed conditions and validated by method Ruggedness shows no significant difference between the analysis carried by two analyst using same experimental conditions and %RSD by both analysts was less than 2.
Limits of Detection and Quantification
The limits of detection (LOD) and quantification (LOQ) were calculated from the relationship between the standard deviation (SD) of the ME linearity and the slope, using the appropriate multiplier. The result reveals that the minimum concentration detected was found to be 0.021 µg/ml, 0.022 µg/ml and 0.026 µg/ml and LOQ be 0.065 µg/ml, 0.064 µg/ml and 0.081 µg/ml for Leaves, seeds and stems extract respectively (Table 8).

CUCURBITACIN-E CONTENT USING VALIDATED METHOD
Using validated method Cu E contents were determined. Comparing the cucurbitacin content by PMA reaction method and by direct UV method indicates that reaction method is more specific than the direct UV method. Cucurbitacin E content in seeds were found to be higher as compared to leaves and stems by both the methods (Table 9).

CONCLUSION

After optimization of the conditions for spectrophotometric determination by direct and PMA reaction UV-Vis spectrophotometric method for determination of cucurbitacin E, all parameters analyzed showed adequate results. The UV-Vis spectrophotometric method described here was successfully validated as suitable for the determination of cucurbitacin E of methanolic extracts of leaves, stems and seeds part of *Cucumis sativus.* This methodology complies with the requirements for analytical use and for ensuring the reliability of the results. The cucurbitacin E content in seeds are found to be higher as compared to the leaves and stems from direct and reaction method. Further PMA reaction was found to be more specific than the direct method.

REFERENCES

1. Jayaprakasam B, Seeram NP, Nair MG. Anticancer and antiinflammatory activities of cucurbitacins from *Cucurbita andreana***.** Cancer Lett. 2003; 189(1):11-16.
2. Tannin-spitz T, Grossman S, Dovrat S. Growth Inhibitory activity of cucurbitacin glucosides isolated from *Citrullus colocynthis* on human breast cancer cells. J. Biochem. Pharmcol. 2007; 73(1): 56-57.
3. Chen JC, Chiu MH, Nie RL. Cucurbitacins and cucurbitane glycosides: structures and biological activities. J. Nat. Prod. Rep. 2005; 22(3): 386-399.
4. Saravanan D, Thirumalai D, Sivakumar A, Asharani IV. A systematic Review of Natural toxins in food plants. Int. j. Res. Ayurveda Pharm. 2016; 7(2): 52-57.

5. Duncan KL, Duncan MD, Allay MC. Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. J. Biochem. Pharmacol. 1996; 52(10):1553-1560.
6. Prashant LK, Hemant RJ, Prasad T, Anantha NN. Cosmetics potentials of herbal extracts. Int. J. Ayur. Pharma Res. 2005; 4: 315-321.
7. Hisahiro K, Masaki B, Toru O. Inhibitory Effect of *Cucumis sativus* on melanin production in melanoma B16 Cells by down regulation of tyrosinase expression. Planta Med. 2008; 74(15): 1785- 1788.
8. Yawalkar KS. Cucurbitaceous or vine crops. In: Vegetable crops of India. Agric. Horticultural Publishing House, Nagpur, India. 1985; 150-158.
9. Jaya D, Swapnil S, Meenakshi A, Sarvesh P. Cytoprotection mediated antiulcer effect of aqueous fruit pulp extract of *Cucumis sativus*. Der Pharmacia Lettr. 2012; 21(3): 61-67.
10. Elisha EE, Twaij HAA, Ali NM, Tarish JH, Alomari MM, Karim S. The anthelmintic activity of some Iraqi plants of the Cucurbitaceous. Pharma Bio. 1987; 2(5): 153-157.
11. Kumar D, Kumar S, Singh J, Vashistha BD, Singh N. Free radical scavenging and analgesic activities of *Cucumis sativus* linn. fruit extract. Journal of Young Pharmacy. 2010; 2(4):365-368.
12. Joysree D, Anusua C, Subrata KB, Utpal KK, Syeda RS, Sheikh ZR. Cytotoxicity and Antifungal Activities of Ethanolic and Chloroform Extracts of *Cucumis sativus* Linn. (Cucurbitaceae) Leaves and Stems. Research Journal of Phytochemistry. 2012; 6(1): 25-63.
13. Mallik J, Akhter R. Phytochemical Screening and In-vitro Evaluation of Reducing Power, Cytotoxicity and Anti-Fungal Activities of Ethanol Extracts of *Cucumis sativus*. International Journal of Pharmaceutical & Biological Archives. 2012; 3(3): 555-560.
14. Sharma S, Dwivedi J, Paliwal S. Evaluation of antacid and carminative properties of *Cucumis sativus* under simulated conditions. Scholars Research Library Der Pharmacia Lettr. 2012; 4 (1): 234-239.
15. Patil MVK, Kandhare AD, Bhise SD. Pharmacological evaluation of ameliorative effect of aqueous extracts of *Cucumis sativus* L. fruit formation on wound healing in Wister rats. Chronicles of young scientists. 2011; 2(4): 207-213.
16. Attard E. Rapid determination of cucurbitacins in Tissues and *invitro* cultures of *Ecballium elaterium* (L.) A. Rich. Cucurbit Genetics Cooperative Report. 2002; 25:71-75.
17. Bruce P, Minkkinen P, Riekkola ML. Practical method validation: Validation sufficient for an analysis method. Microchim. Acta 1998; 128: 93–106.
18. International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. In Q2B(R1): Guideline on Validation of Analytical Procedure- Methodology; ICH Steering Committee: Brussels, Belgium, 2005.
19. Vogel AI. Química Analítica Quantitativa, 6th ed.; Afonso JC, Aguiar PF, Alemcastro RB. Eds.; LTC: Rio de Janeiro, Brazil. 2002; 462.

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