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DETERMINATION OF TOTAL POLYPHENOLS FROM Eclipta prostrata (L.) Hassk. Asteraceae BY FOLIN-CIOCALTEU METHOD

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Abstract

Eclipta prostrata (L.) Hassk. Asteraceae is a universal tree found in Vietnam. It was necessary to develop an analytical method to determine the content of total polyphenols (TP) in this herbal drug. The pre-analytical method was standardized for analysis time, wavelength, and the standard to use. The optimum conditions were: 45 min; 760 nm; Gallic acid respectively. Under these conditions, validation by UV/Vis spectrophotometry proved to be reliable for TP of the crude extracts (CE) from the dried aerial parts *Eclipta prostrata* (L.) Hassk. Asteraceae. Standardization is required for every herbal drug, and this method proved to be linear, precise, accurate, reproducible, robust, and easy to perform.

The results showed that the contents of total polyphenols (TP) in some samples of *Eclipta prostrata* CE are about over 20mg/g extract (calculated on the drying weights)

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Key words: Eclipta prostrata (L.) Hassk. Asteraceae; total polyphenols; UV/VIS spectroscopy validation

1. Introduction

Phenolic compounds, ubiquitous in plants are an essential part of the human diet, and are of considerable interest due to their antioxidant properties. These compounds posses an aromatic ring bearing one or more hydroxyl groups and their structures may range from that of a simple phenolic molecule to that of a complex high-molecular weight polymer. Some of these by-products have been the subject of investigations and have proven to be effective sources of phenolic antioxidants [1], [2].

This paper deals with the analytical procedure to determine the total polyphenols (TP) from the Eclipta prostrata (L.) Hassk. Asteraceae CE by Folin - Ciocalteu method [1],

2. Experimental methods

Reagents and standards

The solvents used were: ethanol (Merck), ethyl acetate (Merck), methanol (Merck ©), the standard gallic acid (99,69%, Fluka ©), the reagent Folin-Ciocalteu -(FCR) (Merck -1.09001), anhydrous sodium carbonate.

Plant material and preparation of extracts

- All the aerial parts (bark, twigs, and leaves) of *Eclipta prostrata* (L.) Hassk. Asteraceae were collected at Long An province in February 2020. After collected, these parts of *Eclipta prostrata* (L.) Hassk. Asteraceae plants were cleaned, cut into small pieces and then dried. They were put in clean package ant stored in the cool dry places. They were grounded for having the suitable sizes to extract by many solvents (distilled water; MeOH; EtOH; EtOAc)

2.1. Preliminary tests

Preparations of the experimental solutions

- Standard solution (solution S): Transfer about 25 mg of Gallic acid (99,69%) w.s., accurately weighed, into a 50 mL volumetric flask, add $\sim$ 10 - 20 mL of distilled water and dissolve in an ultrasonic bath. Dilute to volume with the same solvent and mix. Transfer 1.0 mL of this solution into a 10 mL volumetric flask, dilute to volume with distilled water and mix. ($\sim$ 50 ppm). This solution was used daily and was preserved in refrigerator at 4 ^{0}C, avoid the light. After carrying out the procedure with FCR, the solutions S were measured the absorbances by the UV-1800 SHIMADZU Spectrophtometer at 760nm.

- Test solution (solution $\mathbf{T}_n$): Weight exactly about 1 g of the *Eclipta prostrata* CE by analytical balance (0.1 mg precision), transferred to a 100 mL Erlenmeyer flask, add 80 mL of distilled water. Ultrasonic agitation at 40 ^{0}C$\pm$

5 in 30 minutes. The content of the flask was filtered through cotton filter and transferred to a 100 mL volumetric flask, the filter cotton was washed with another 15 mL of distilled water. Then the volumetric flask was completed to the mark for having the stock test solution. Filter through paper, reject about 20-30 mL first filtrate. After carrying out the procedure with FCR, the solutions T were measured the absorbances by the by the UV-1800 SHIMADZU Spectrophtometer at 760nm.

2.1.1. Determination of the maximum wavelength

3 volumetric flasks of 10mL were prepared for studying of the maximum wavelength of the phenolic compounds derivatives which were formed with FCR.

Prepare the solutions for determining of the maximum wavelength

Add into the 1st flask (Blank solution): 1mL distilled water; 2nd flask (Standard solution): 1mL solution S; the 3rd flask (Test solution): 1mL solution T. Then, 5mL of 10% F-C reagent; 1.5 mL Na_2CO_3 20% were added to the 3 flasks and add the distilled water to the mark. Mix well. Filter through paper, reject about the 1-3 mL first filtrate. The rest was used for scanning by UV- Vis spectrophotometry from 400 - 900nm for determining the maximum wavelength.

Table 1: Preparation of the samples for determining the maximum wavelength

Volumetric Flask of 10 ml	Blank solution	Solution S	Solution T
Solution T (ml) (water)	0	0	0.2
Solution S (ml)	0	0.2	0
10% FCR (ml)	5	5	5
20 % Na_2CO_3 (ml)	1.5	1.5	1.5
Distilled water q.s.p (ml)	10	10	10

Wait for 40 minutes and measure the absorbances at 760 nm. Parallel measurement with blank solution

2.1.2. Determination of the volume ratios (10% FCR and Na2CO3 20% solutions)

5; 6; 7 mL of the 10% FCR was added, respectively, to 9 volumetric flasks of 10 mL. Then, add 1; 1.5; 2 mL of Na2CO3 20% solution as presenting in table 1. Complete to the mark with distilled water.

2.1.3. Determination of the solution T volume

0.1; 0.2; 0.3; 0.4; 0.5; 0.6; 0.7; 1 ml of T solutions were added respectively into 8 volumetric flasks of 10ml. Add exactly 5mL of 10% FCR solution, 1.5

ml of 20% Na_2CO_3 solution. Complete to the mark with distilled water as presenting in table 1. Wait for 40 minutes and measure the absorbances at 760 nm. Parallel measurement with blank solution

2.1.4. Determination of the chemical reaction time

- Prepare the solution S (and solution T)

0.4 ml of solution S (or 0.5 ml solution T) was pipetted into the 10ml volumetric flask. Add exactly 5mL of 10% FCR, 1.5 ml of 20% Na_2CO_3 solution. Complete to 10ml with distilled water. Mix well as presenting in table 1. Read the absorbances at λmax =760 nm after the short period of time as 15 minutes

2.2. Validation of analytical procedure

The UV-spectrophotometry method was validated according to official validation guidelines : ICH Guidelines (ICH Q2R1) for Analytical Procedure and Validation.

2.2.1. Suitability

- For the Standard solution: prepare the solution S as part of 2.1.4. Determination of the chemical reaction time. Measuring the absorbance of this solution in 6 times continuously.

2.2.2. Specificity (selectivity)

The specificity was assessed by testing analytical interferences. This analytical parameter was determined by comparing ultraviolet absorption spectra obtained from TP of standard solution, sample solution and placebo. The spectra were obtained in the range of 2 ppm to 12 ppm, and the overlap of absorption bands was evaluated. Through spectral scans, it was also possible to determine the absorption wavelength maxima of TP when solubilized in distilled water.

2.2.3. Linearity

The method linearity was studied by performing three independent analytical curves, within five concentration levels ranging from 2 ppm to 12 ppm μg/mL. Standard plots (concentration versus absorbance) were constructed, and linearity was evaluated statistically by linear regression analysis through least square method and applying ANOVA (analysis of variance).

2.2.4. Precision

Precision was determined by repeatability (intraday). Repeatability was evaluated by assaying six samples solutions at 2.0 μg/mL during the same day. The analyses were done in triplicate and results were expressed as the relative standard deviation (RSD) of the analytical measurements. Samples were prepared described.

2.2.5. Accuracy

Accuracy was determined based on the recovery of known amounts of Gallic acid reference standard added to samples at the levels of 80, 100 and 120% of the sample concentration (2.0 μg/mL). The accuracy was calculated as the percentage of the mass recovered and also expressed as the relative standard deviation (RSD) between the measurements.

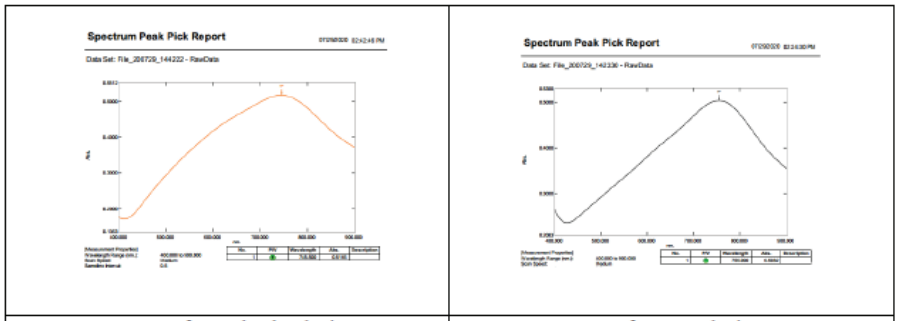
nghi**Statistical analysis**

All absorbance values, relative to the reaction mixtures tested, were evaluated statistically by analysis of the variance using one-way ANOVA ($p \leq 0.05$).

3. Results and discussions

3.1. Results of Preliminary tests

3.1.1.Determination of the maximum wavelength



UV- vis spectre of Standard solution | *UV- vis spectre of* Test solution

Figure 1: The UV- vis spectra of Standard and Test solutions

- Comment: Both the Standard solution (solution S) and the Test solution (solution T) have the maximum wavelengths in the distance from 745 nm to 760 nm.

Continue to mesure the absorbances of Standard solution and Test solution at 745 nm, 755 nm and 760 nm for choosing the maximum wavelength.

Table 2: Results of determining the maximum wavelength

Absorbances	745 nm	755 nm	**760 nm**
Standard solution (solution S)	0.5138	0.5124	**0.5109**
Test solution (solution T)	0.5005	0.5023	**0.5016**

Comment: Absorbances at 3 wavelenghts 745 nm, 755 nm and 760 nm are the same. There fore, 760 nm was chosen the maximum wavelength as many references which studied by Folin- Ciocalteu method [2].

3.1.2. Determination of the volume ratios (10% FCR and Na2CO3 20% solutions)

Table 3: Study on the volume ratios of (10% FCR and Na2CO3 20% solutions)

STT	10%FCR (ml)	20% Na_2CO_3 (ml)	Absorbances	
			Standard solutions	Test solutions
1	5	1	0.5804	0.4755
2		**1.5**	**0.5950**	**0.4775**
3		2	0.5480	0.4157

- Comment: The volumetric ratio of the 10% F-C reagent and Na2CO3 20% solution was chosen: 5:1.5.

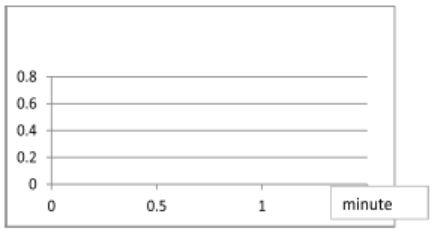
3.1.3. Determination of the solution T volume.

Table 4 : Study on the volumes of solution T

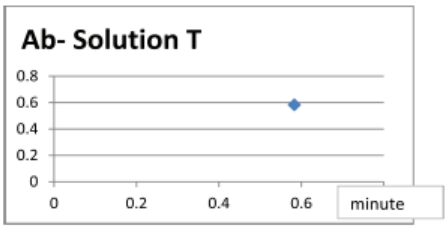
Flasks	1	2	3	4	5	6	7	8
mL (solutionT)	0.1	0.2	0.3	0.4	**0.5**	0.6	0.7	1
A_{760}	0.185	0.325	0.474	0.628	**0.763**	0.899	1.229	1.545

- Comment: The volume 0.5 mL of solution T (2 mg/ml) was choosen because this absorbance is between the range and it's easy to carry out the experiment.

3.1.4. Determination of the chemical reaction time.



UV- vis spectre of Standard solution



UV- vis spectre of Test solution

Fig.2: Determination of the chemical reaction time of Solution S and T

Table 5: Results of study on the chemical reaction time of Solution S and T (760nm)

During period (minutes)	Absorbances of solution S + FCR	Absorbances of solution T + FCR
15	0.5699	0.4507
30	0.5835	0.4607
45	**0.5836**	**0.4633**
60	0.5832	0.4586

Comment: the results of studies on the chemical reaction time showed that after 45 minutes, the absorbances of Standard solutions and Test solutions are stable. Therefore, 45 minutes was chosen the period for measuring the absorbances at 760nm.

3.2. Validation of analytical procedure

3.2.1. Suitability

Table 6: Study on the suitability system

N_0	1	2	3	4	5	6	
Ab	0.5831	0.5830	0.5830	0.5830	0.5829	0.5830	RSD%=0.011

Comment: the results of study on the **Suitability was met the requirement of ICH** RSD% ≤ 2%.

3.2.2. Specificity (selectivity)

Representative UV spectra of Gallic acid standard reference, test solution are presented in Figure 2, at the wavelength selected for analysis (760 nm), it is possible to observe a same absorbance from Gallic acid reference standard and test solution. Experimentally, this performance indicates that there is no interference in the analysis. The method is specific for the intended analysis.

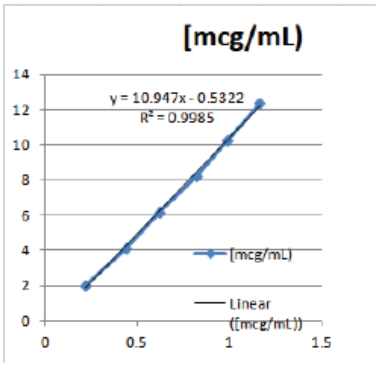
3.2.3. The linearity

For Standard solution (solution S)

The linearity of standard solution was investigated in water and gallic acid, respectively, at seven concentration levels in a range between 20; 40; 60; 80; 100; 120 μl of solution S, corresponding to approximately 2 ppm to 12 ppm of Gallic acid Add exactly 5mL of 10% FCR; 1.5 ml of 20% Na_2CO_3 solution. Completed to 10ml with distilled water. Read the absorbances at λmax =760 nm after 45 minutes.

Table 7: Results of study on the Linearity of solution S

Flask	1	2	3	4	5	6
C (ppm)	2,06	4,12	6,18	8,24	10.3	12,36
Dilution	25	12,5	8	6	5	4
A_{760}	0.2202	0.4352	0.6176	0.8183	0.9892	1.1630



Comment:

The calibration curve was linear over the concentration range of 2 ppm - 12 ppm for standard solution with linear equation: y = 0.0912x + 0.0496;

Correlation coefficient (r) of 0.9985 . This result meet the requirement of ICH in linearity RSD% ≥0.998

Fig. 3: Linear regression plot of solution S (2 ppm to 12 ppm).

3.2.4. The Repeatability

Repeatability was evaluated by analysing six dependent test solutions. Prepare the 6 solution T as part of 2.1.4. (Determination of the chemical reaction time). Measuring the absorbances of theses 6 solution continuously for calculating the content of total phenolic compounds (mg/1g extract)the . RSD% $\leq$ 2%.

Table 8. Results of study on the Repeatability of Absorbances in samples (n = 6)

No of solutions T	1	2	3	4	5	6	Solution S
Weights (g)	0.7929	0.7877	0.7921	0.7832	0.7902	0.7910	0.0252
Ab. of test solutions	0.4973	0.5012	0.5016	0.4995	0.5005	0.5020	0.5163
Content of TP (mg/1g extract)	30.52	30.96	30.81	31.03	30.82	30.88	Average: 30.84

Comment: RSD = 0.58% was accepted due to < 2%

X_{av} = 30.84 % ; SD = 0.176; RSD = 0.58 (%) ; e = ± 0.19 (%) ; μ = 30.84 ± 0.19 (%)

3.2.5. Accuracy

Prepare 3 level different concentrations 80%; 100% and 120% versus the concentration of quantitation of solutions S. Then, carry out as the applicated procedure as table 1.

Table 9: Results of Recovery of 3 different levels of Gallic acid standard

Concentration level	Concentrations of Gallic acid added (mg/ml)	Concentrations of Gallic acid found (mg/ml)	Recovery (%)	Average (%)
80%	24	23,83	99,30	
	24	23,46	97,76	98,40
	24	23,56	98,15	RSD%: 0.81
100%	30	29,36	97,87	
	30	29,63	98,77	98,32
	30	29,50	98,32	RSD%: 0.46
120%	37	36,53	98,74	
	37	36,18	97,78	98,69
	37	36,84	99,55	RSD%: 0.90

Comment: Accuracy express the recovery between the added values and the found values. The results obtained showed that a high accuracy of the

applicated procedure. Mean recovery of each concentration were [96- 102%]. So, this procedure conform the accuracy.

3.3. Application

Prepare the extractions: For having many other crude extracts , distilled water was replaced by other solvents such as MeOH; EtOH 96%; EtOAc for comparison of performance % of the content of TP in these crude extracts.

Table 10: Performance of TP in extracts by solvents

	Weight mass (g)	**Residual mass (g)**	Performance (%)
H_2O	1.0030	0.2528	25.20
MeOH	1.0028	0.1609	16.05
EtOH 96%	1.0029	0.1530	15.26
EtOAc	1.0067	0.0411	4.08

Analytical procedure: prepare the solutions for measuring as Table 11.

Table 11: Preparation the samples for measuring UV Vis

Flask of 10 ml	Blank solution	Solution S	Solution T
Solution of difference extracts (ml)	0	0	0.2
Solution S (ml)	0	0.2	0
10% FCR (ml)	5	5	5
20 % Na_2CO_3 (ml)	1.5	1.5	1.5
Distilled water q.s.p (ml)	10	10	10

Close the cover, mix well each volumetric flasks. The chemical reactions take place at room temperature about 45 minutes before measuring the absorbance. Read the absorbance at 760nm in the 1cm cuvet, parallel with the blank solution.

Calculation:

$$\%P = \frac{A_T}{A_S} \times C_C \times \frac{500}{M} \times \frac{C\% \times 100}{(100 - h)}$$

%P: the content of total phenolic compounds in the Eclipta prostrata (L.) extracts;

M : Mass of *Eclipta prostrata* **(L.) Hassk. Asteraceae extracts** (g);

A_T : Absorbance of Test solution.

A_C : Absorbance of Standard solution.
C_C: Concentration of standard solution S (mg/ml);
h : Humidity of *Eclipta prostrata* **(L.) Hassk. Asteraceae extracts**;
C%: purity of Gallic acid.

Table 12: The content of TP (mg/1g extract) in difference extracts

Extract of	Mass of extract (g)	Dilution	Absorbances	The content of TP (mg/1g extract)
Water	0.7929	5000	0.5019	26.01
MeOH	0.6242	2000	0.4183	11.01
EtOH 96%	0.4562	2000	0.3212	11.57
EtOAc	0.1053	200	0.5509	8.60
$CHCl_3$	0.1114	200	0.4879	7.20

Comment: The content of TP in water extract is the highest (26,01mg/1g). Therefore, water was chosen when determining the solvent for extracting.

3.4. Discussion

UV-visible spectrophotometry is an analytical technique widely used in the quality control of drugs, being present in official monographs, for identification and quantitation [2]).

Although there are limitations regarding specificity, the UV-spectroscopy presents some advantages when compared to chromatographic methods, such as faster analysis, low operating costs and low generation of waste. An alternative for improve the specificity and sensitivity of the technique is to perform UV derivative spectrophotometric method [2].

These advantages are determinants for new analytical investigations with quantitative focus. In the present study, the method development started with preliminary tests, applied to establish better conditions for analyses, studying the solvents for sample extraction, the concentration for analyses and the ?max for quantification. In order to determine the best wavelength for analysis, scans were performed in the range of 200 to 700 nm, whose results indicated the maximum absorbance at 760 nm. Due the high solubility in water and based on studies already published in the literature, we selected water and methanol as solvents, evaluating the extraction in different times by ultrasonic bath. Samples submitted to the extraction with Ethyl acetate presented turbidity and resistance to filtration, and the results illustrated a higher variation in the content of TP after extraction.

4. Conclusion

The proposed method for TP of *Eclipta prostrata* (L.) CE has many advantages over other analytical methods due to its rapidity, lower cost, environmental safety and better sensitivity. The method can be successfully employed for TP quantification in all parts of *Eclipta prostrata* (L.) samples as leaves, flowers, roots... etc. The results showed that the contents of TP in *Eclipta prostrata* (L.) water CE are about 26,01 mg/g extract (calculated on the drying weights of extracts)

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