THE COMPARISON OF TWO COLORIMETRIC METHODS FOR DETERMINATION OF TOTAL TANNIN IN *Psidium guajava* L. LEAVES BY FOLIN-CIOCALTEU’S AND 1,10-PHENANTHROLINE REAGENTS

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ABSTRACT

Objectives: Tannin was important metabolite secondary in plants. So the analytical method of tannin have to validated as standard method. *Psidium guajava* L. leaf containing a lot of tannin, so it was use for this research.

Methods: Comparison parameters validation (linierity, LOD, LOQ, accuracy, and precission) of colorimetry for determination tannin in *Psidium guajava* L leaf by 1,10 phenanthroline and Follin Ciocalteu’s reagents.

Results: Parameter validation for 1,10 phenanthroline and Follin Ciocalteu’s reagents respectively were: LOD 0.47µg/mL, 1.48µg/mL; LOQ 1.57µg/mL, 4.94µg/mL; linierity 0.9994, 0.9993; accuracy as % recovery 87.85±3.79 %, 32.39±8.53 %, and precission as coefficient of variance 4.3%,26.42%.

Conclusion: Colorimetry method of total tanin in *Psidium guajava* L leaf by 1,10 phenanthroline reagent had better validation than Follin Ciocalteu’s reagent.

Keyword : Tannin, *Psidium guajava* L leaf, 1,10 phenanthroline, Follin Ciocalteu’s reagent, validation

INTRODUCTION

Tannins are very important commercial products. However, their chemistry is very complex and diverse. Tannin is one of the most secondary metabolite in *Psidium guajava* L. leaf. Various gravimetric, volumetric and colorimetric Methods for the determination of tannins appear in the literature. The most widely used methods are the volumetric permanganate method developed by Lowenthal, after some modification. The volumetric permananate method has been criticised because of the errors involved in the
determination of the end-point. Two colorimetric methods for determination of total tannin in plants were 1,10-phenanthroline and Folin-Ciocalteu’s Reagent (FCR) methods.

In this study, determination of total tannin in *Psidium guajava* L. leaf by 1,10-phenanthroline Reagent colorimetric method was compared by Folin-Ciocalteu’s Reagent colorimetric method.

**MATERIALS AND METHODS**

**Sample preparation:** *Psidium* leaves collected, dried in a dark place at home temperature. Dried leaves were powdered using an electric device until it passed through a 40 mesh sieve.

**Chemicals:** Folin-Ciocalteu reagent, sodium carbonate, aquadest, tannic acid as reference, casein, gelatine, 1,10-phenanthroline, methanol, CH3COONa, FeCl3.

**Methods:** Extract preparation for determination of total tannin by FCR method was done with boiling *Psidium guajava* L. leaves powder in methanol 30% at 70°C for 15 minutes. An aliquot of the extract was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water at 1:10 v/v) and 4 ml (75 g/l) of sodium carbonate. The tubes were vortexed for 15 sec and allowed to stand for 30 min at 40°C for color development. Absorbance was then measured at 745 nm using the Hewlett Packard UV-VS spectrophotometer. In this method, tannin was precipitated by casein and absorbance was measured at 745 nm. Total phenolic compounds was calculated using a standard curve prepared with dilutions of tannic acid. Total tannin was measured based on difference between absorbance of extract with and without addition of casein. Percentage of recovery was made by addition standard method with tannic acid as reference.

Extract preparation for determination of total tannin by 1,10-phenanthroline method was done with boiling *Psidium guajava* L. leaves powder in aqua dm for 1 hour, filtrated and add to 100.0 mL. An aliquot of the extract were added 2.5 mL FeCl3 and aqua dm, heated in 80°C for 20 min. Solution then added 2.5 mL CH3COONa in order to keep the solution within a range from 3 to 4 pH values, 5 mL of 0.5% o-phenanthroline,
0.5 mL EDTA solution, and aqua dm were added until a 25.0 ml final volume was obtained. Absorbance was measured at 510 nm. Precautions were taken so the solution to be measured and the corresponding control showed the same pH values. In this method, tannin was precipitated by gelatine and absorbance was measured at 510 nm. Total tannin was measured based on difference between absorbance of extract with and without addition of gelatine. Percentage of recovery was made by addition standard method with tannic acid as reference.

**RESULT AND DISCUSSION**

The Folin Ciocalteu reagent consists of 100 ml of sodium tungstate dihydrate, 25 of sodium molybdate dihydrate, 50 ml of 85% phosphoric acid solution and 100 ml of 36% hydrochloric acid solution. The Folin reaction is based on the reduction of phosphomolybdiate acid by phenols of tannin in aqueous alkali. This method determines the total free phenolic hydroxyl groups and is therefore a method used to determine total soluble phenolics.

Phenanthroline is a sensitive reagent for the determination of Fe2+, it can react with Fe2+ to form an orange complex in acidic solution. A new spectrophotometric method was proposed for the determination of polyphenols in samples based on polyphenols could react with Fe3+ to produce Fe2+, and the orange complex would be formed by the reaction of Fe2+ with Phenanthroline in acidic solution.

Protein precipitation are often cited as being more realistic for estimating the content of tannins in plants because these methods are more closely related to biological effects (Martin and Martin, 1982; Hagerman, 1987). The methods that rely on the precipitation of protein from solution usually determine the amount of protein precipitated or the amount of phenolic compounds that remain in solution.
A. Calibration Curve of Tannic Acid by Folin-Ciocalteu’s Reagent

B. Calibration Curve of Tannic Acid by 1,10-Phenanthroline Reagent

C. Percentage of Recovery of Tannin by Folin-Ciocalteu’s Reagent in Psidium guajava leaves
D. Percentage of Recovery of Tannin by 1,10-Phenanthroline Reagent in *Psidium guajava* leaves

<table>
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Mean ± SD RSD: 32.39± 8.53% 26.42%

CONCLUSION

Colorimetric method for determination of total tannin in *Psidium guajava* L. leaves by 1,10-phenanthroline reagent had better parameter of validation than by Folin-Ciocalteu’s reagent

REFERENCES

