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Determination of Total Flavonoid Content from Ethanolic Leaf Extract of *Lagenaria siceraria* Lin.

			
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ABSTRACT

The *Lagenaria siceraria* leaf is one of the economical source of flavonoid especially quercetin. The total flavonoid content is usually determined spectrophotometrically using Ultraviolet spectroscopy. The collected plant was subjected to soxhlet extraction and the collected extract was dried by vacuum evaporator. The dried leaf extract was taken for determination of total flavonoid content. Plant material was collected and dried under shade, the dried grounded leave powder extraction with ethanol in soxhlet extractor an about 74 hours. The standard quercetin was weighed and dissolved in Dimethyl sulfoxide (DMSO) and further dilution (20 mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) made with DMSO. The collected and evaporated extract dissolved in DMSO and dilution was made with the DMSO. From the above solution from Standard and Test solution were taken for the incubation with equal volume of 2% $AlCl_3$ at ambient temperature about 10minutes and measure the absorbance spectrophotometrically at 435nm. The accuracy of method validates my made serial dilution of Standard Quercetin and calibration curve plotted with the absorbance Vs concentration of Standard Quercetin value obtained from spectrophotometrically (tab.1). The linearity of curve calculated by correlation coefficient method and the R^2 value is 0.9726. The coefficient value near to the +1 indicates the good linearity of the graphical value shows the accuracy of the method. The total flavonoid content of *Lagenaria siceraria* leaves extract was determined by spectrophotometrically and the obtained value shows significant amount of flavonoid present in *Lagenaria siceraria* leaves.

INTRODUCTION

The *Lagenaria siceraria* most common vegetable plant in tropical and Sub-tropical area, it belonging to the family cucurbitaceae, possess more reliable active constituent. the amino acid composition of the edible material is as follows leucines (2.3 mg/gm), phenylalanine (1.0 mg/gm), valine (1.0 mg/gm), tyrosine (0.4 mg/gm), alanine (1.0 mg/gm), threonine (0.8mg/gm), glutamic acid (1.7 mg/gm), serine (0.9 mg/gm), aspartic acid (2.9 mg/gm), cysteine (1.7mg/gm), cystenine (0.4 mg/gm), lysine (5.2 mg/gm), methionine sulphoxide (0.3 mg/gm) and proline (0.3mg/gm). (1) Pharmacologically the leaves have more medicinal value, the various of research shows different pharmacological activity like, Anti-hyperglycemic activity(2), anthelmintic activity(3), Cardioprotective effect(4), Anticancer Activity(5), antihyperlipidemic(6), immunomodulatory activity(7) and the leaf juice used for baldness(1).

MATERIALS AND METHODS

Plant

The dried and grounded leaves of *Lagenaria siceraria* were collected from Rettanai village, Villupuram district in the month of August, 2016



Chemicals and reagents

Ethanol (95 %)

Dimethyl sulfoxide solvent purchased from

Aluminium chloride (2%) -2gm w/w dissolved in demineralized water

Demineralized water

METHODS

Preparation of plant Extract

The collected leaves were dried in shade. Prior to drying, the leaves were washed with DM water to remove foreign matter and ground. Accurately weighed 100gm leaf powder packed in 250 ml Soxhlet extractor using Ethanol (95 %) as a solvent. The process was continued for about 74 hours, and until the solvent dropping down from extractor into the RBF appeared colourless. Once the extraction was over, the setup was dismantled and extract was collected dried in vacuum evaporator.

Preparation of Standard Stock solution

Accurately weighed 25mg of Quercetin standard transferred to 100ml of volumetric flask and dissolved in dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100mcg) were made with dimethyl sulfoxide.

Preparation of Test Solution

The leaf extract was weighed accurately equal to the weight of Standard Quercetin and transferred to 100ml volumetric flask and the extract dissolved in dimethyl sulfoxide (DMSO). The dilution was made with dimethyl sulfoxide.

Procedure

From the prepared solution of standard and test solutions, 2ml was withdrawn from each concentration to the test tube and added equal volume of 2% Aluminium Chloride solution to every single concentration. Incubate the solution about 10minutes at ambient temperature. After 10minutes, Standard and sample solution measure the absorbance of spectrophotometrically at 435 nm with the standard and test sample solutions.

RESULT AND DISCUSSION



The determination of total flavonoid content of Lagenaria leaves were performed with the Quercetin standard. The accuracy of test was made by the serial dilution of Standard and absorbance was measured spectrophotometrically at 435 nm (Tab.1). The obtained data were plotted as a Standard Calibration curve (Fig.1).

Table 1: Spectrophotometric absorbance of Standard and Sample

Sr. No.	Concentration of standard solution($\mu\text{g/ml}$)	Absorbance(435nm)
1.	20	0.135
2.	40	0.175
3.	60	0.213
4.	80	0.306
5.	100	0.347

Standard Calibration Curve for Quercetin

To determine the accuracy of the flavonoid compound the calibration curve done by made serial dilution (20mcg, 40mcg, 60mcg, 80mcg, 100mcg) of quercetin Standard stock solution, the absorbance plotted against concentration (Fig.1)

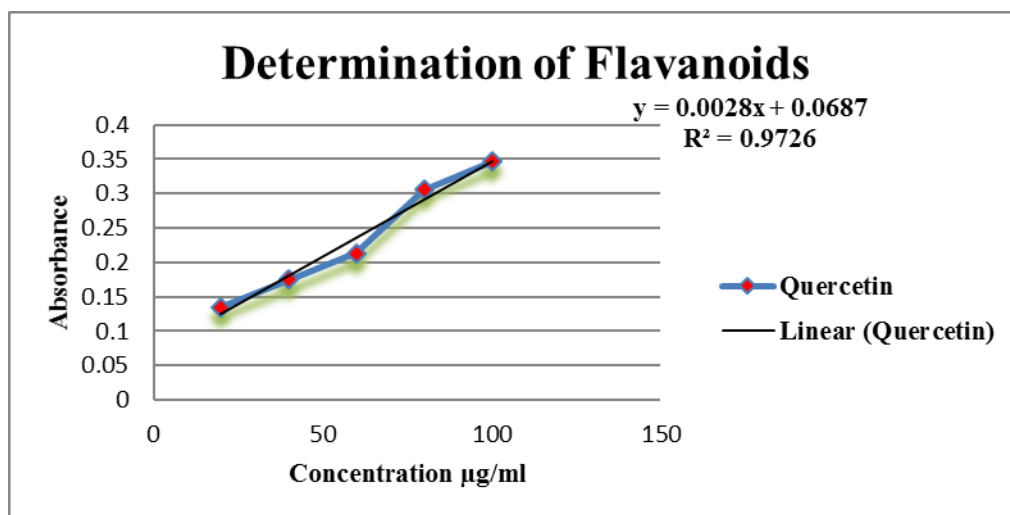


Fig.1: Standard Calibration Curve for Quercetin

The graphical value



The values taken from the above graph was subjected to statistical analysis. The correlation coefficient R^2 value was calculated. This is done in order to check out the linearity of the experimentally obtained data. Hence the calculated $R^2 = 0.9726$ indicates a good linearity in the curve.

Determination of Concentration of flavonoid

From the replicate absorbance value obtained by the spectrophotometry, the calculation of concentration of flavonoid present in 1gm of the extract was calculated by applying the dilution factor and the 1gm of extract contain 40mg/gm of flavonoids. From the spectrophotometric analysis of *Lagenaria siceraria* leaf extract contains 40mg/gm of flavonoids.

CONCLUSION

The standard calibration curve shows the correlation coefficient value $R^2 = 0.9726$. The value near to the 1, indicates positive correlation between the concentration and absorbance. Hence

from the obtained data, the spectrophotometric of *Lagenaria siceraria* leaves extract proved to contain flavonoid compound in considerable amount.

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