DETERMINATION OF TOTAL FLAVONOID CONTENT IN ETHANOLIC LEAF EXTRACT OF *Moringa Oleifera*

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ABSTRACT

*Moringa oleifera*, a small genus of quick-growing trees distributed in India, Arabia, Asia Minor, Africa. It belongs to the family Moringaceae. Two species are recorded from India, of which one, *M.oleifera*, is widely cultivated in the tropics for its edible fruits. With a synonym: *Moringa pterygosperma*, it is very popular in the Southern kitchen. Commonly, it is called the Drumstick tree or the radish tree. Ethno-medicinal use of the various plant parts are as follows: ROOT- Rheumatism, asthma, liver complaint, Paralysis, epilepsies, scorpion bite, snlue bite, tooth ache, astringent. GUM- Ear complaint. BARK- Stomach trouble, fever, eczema, typhoid, boils, toothache, poisonous bites, rheumatism. LEAF- Scurvy, wound, night blindness, diarrhea, dysentery, cough, cold, diseases of liver, spleen. FLOWERS- Eye ailments. PODS- Eye ailments, diseases of Liver, spleen. SEED- Indigestion, Seed oil in Gout, Rheumatism. The total flavonoid content is usually determined spectrophotometrically using Ultra-violet 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. Dimethylsulfoxide was used as the solvent. The absorbance was measured at 435nm.

KEYWORDS: *Moringa oleifera*, Moringaceae, Ethnomedicinal use, Total Flavonoid Content, 435 nm.

INTRODUCTION

The tree is indigenous to North-West India and is plentiful on recent alluvial land in or near sandy beds of river and streams. It belongs to the family Moringaceae. The leaves are rich in
carotene and ascorbic acid. Analysis gave the following values: moisture-75.0, proteins-6.7, fat(ether extract)fat-1.7, carbohydrates-13.4, fibre-0.9, and mineral matter-2.3%, calcium-440, phosphorous-70, iron-7.0 mg/100g, copper-1.1 μg/g, and iodine 51 μg/g are present. Leaves contain carotene 11,300 i.u, vitamin B1 210 μg, nicotinic acid 0.8 mg, ascorbic acid- 220 mg, tocopherol 7.4 mg/100g.

The essential amino acid present in the leaf proteins are (g/16g N): leucine (9.3), phenylalanine (6.4), valine (7.1), arginine (6.0), histidine (2.1), lysine (4.3), tryptophan (1.9), methionine (2.0), threonine (4.9), leucine (9.3), isoleucine (6.3).

MATERIALS AND METHODS

Plant material
The leaves of Moringa were collected from the domestic gardens of Chennai in the month of August, 2016. They were washed thrice with water to remove any earthy matter and dried under shade for a week or so. Then, the dried leaves were ground into a coarse powder.

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
Accurately weighed 100gm leaf powder packed in 250 ml Soxhlet extractor. The extraction was run 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 SAMPLE
Preparation of Standard Stock solution
Accurately weighed 25 mg of Quercetin standard transferred to 100 ml of volumetric flask and dissolved with dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg, 80mcg, 100 mcg) 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 100 ml volumetric flask and the extract dissolved with dimethyl sulfoxide (DMSO). The serial dilution (20mcg, 40mcg, 60mcg, 80 mcg, 100 mcg) were 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 10 minute at ambient temperature. After 10 minute, measure the absorbance spectrophotometrically at 435 nm with the standard and test sample solutions.

RESULT AND DISCUSSION
The determination of total flavonoid content of Moringa leaves were performed with the Quercetin standard. The accuracy of test were 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>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of standard solution(µg/ml)</th>
<th>Absorbance (435nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>20</td>
<td>0.136</td>
</tr>
<tr>
<td>2.</td>
<td>40</td>
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</tr>
<tr>
<td>3.</td>
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</tr>
<tr>
<td>4.</td>
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<td>0.309</td>
</tr>
<tr>
<td>5.</td>
<td>100</td>
<td>0.353</td>
</tr>
<tr>
<td>7.</td>
<td>Sample</td>
<td>0.148</td>
</tr>
</tbody>
</table>

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

Concentration calculation
From the absorbance value obtained by the spectrophotometry, the calculation of concentration of flavonoid present per milli litre of the extract was calculated by applying the dilution factor. It was found out as 0.7073 mg/ml of the extract.

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 *Moringa oleifera* leaves extract proved to contain flavonoid compound in considerable amount.

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