

Quantitative Estimation of β -Sitosterol, Total Phenolic and Flavonoid Compounds in the Leaves of *Moringa oleifera*

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Abstract: A rapid method has been developed for the quantitative estimation of β -sitosterol, total phenolic and flavonoid compounds present in the leaves of *Moringa oleifera* Lam. (Family: Moringaceae). The hydroalcoholic extract of the leaves was subjected for the quantitative estimation of these compounds. The hydroalcoholic extract of the leaves of *Moringa oleifera* was prepared by the process of cold maceration using ethanol and water, followed by distillation. The extract prepared was tested for preliminary qualitative phytochemical screening, followed by the quantitative estimation of β -sitosterol by LC-MS and total phenolic, flavonoid compounds by Visible-Spectroscopy. The reports revealed the presence of 90mg/g of β -sitosterol, 8 μ g/mL and 27 μ g/mL of total phenolic and flavonoid compounds respectively in the leaves of *Moringa oleifera*. Thus, the therapeutic potential of *Moringa oleifera* may be due to presence of these major phytoconstituents.

Key words: *Moringa oleifera*, β -sitosterol, phenolic compounds, flavonoids, LC-MS, Visible-Spectroscopy.

Introduction and Experimental

Moringa oleifera (Family: Moringaceae) known as Drumstick tree in English has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of various important minerals and are a good source of proteins, vitamin A, C and E, β -carotene, amino acids and various poly phenolics¹. The *Moringa* plant is a rich and rare source of combination of zeatin, quercetin, β -sitosterol, caffeoylquinic acid, Kaempferol, kaempferitrin, Isoquercitrin, rhamnnetin, rhamnase and also in a fairly unique group of compounds called glucosinolates and isothiocyanates. The leaves are also rich source of essential amino acids such as methionine, cystine, tryptophan, and lysine with a high content of proteins². The extracts of leaves, seeds and roots of *Moringa oleifera* have been extensively studied for many potential uses including wound healing, anti-tumour, anti-fertility, hypotensive and

analgesic activity, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, hypocholesterolaemic, antifungal, antibacterial, antifungal, antioxidant etc., The paste of the leaves is used as an external application for wounds³. *Moringa* leaves are a good source of natural antioxidants. Very few methods have been reported for the estimation of different constituents present in *Moringa* plant. As per the literature review, there is no experimental evidence presently available with regard to the quantification of β -sitosterol^{4,5}. Hence, the present study was carried out in an attempt to quantify the amount of β -sitosterol, total phenolic and flavonoid compounds in leaf extract of *Moringa oleifera*.

β -Sitosterol (purity: 98.91%), Gallic acid monohydrate (purity: 99.97%) and Rutin (purity: 99.81%) were obtained from SD Fine Chem. Ltd. (Mumbai, India), Methanol of HPLC grade is obtained from E.merck (Mumbai, India) and all other chemicals used were of

analytical grade. Purified water from Milli-Q-system (Millipore, Bangalore, India) was used throughout the analysis. Analysis is carried out on Shimadzu LC-MS 2010A and Shimadzu 1700A UV-VIS spectrophotometer.

Preparation of hydroalcoholic extract⁶

The leaves of the plant *Moringa oleifera* were shade dried, milled, and ground into coarse powder with the help of a mixer. The powdered material was subjected to cold maceration using sufficient quantity of ethanol and distilled water (1: 1) for 10 days with intermittent shaking in a round bottomed flask. On 10th day, it was strained and marcs were pressed. The expressed liquids were added to the strained liquids and the combined liquids were clarified by filtration and the filtrate was subjected to distillation at temperature 60 °C for removing ethanol and water. After distillation, the semi solid obtained was kept in a vacuum desiccator for drying. The percentage yield of the extract was 13.7%w/w.

Quantification of β -Sitosterol in the leaf extract⁷

Preparation of standard stock solution

10mg of β -Sitosterol working standard was accurately weighed and transferred into a 10mL volumetric flask and dissolved in a mixture of methanol and chloroform (1:1). The volume was made up to the mark using the same solvent to give a concentration of 1mg/mL. The solution was labeled and stored in a refrigerator below 8°C. The above solution was suitably diluted for further analysis.

Preparation of sample solution

10mg of extract was transferred into a 10 mL volumetric flask and it was dissolved in a mixture of methanol and chloroform (1:1). The volume was made up to the mark with the same solvent to give a concentration of 1mg/mL. This solution can be labeled and stored in a refrigerator below 8°C and suitably diluted at the time of analysis.

Various trials were carried out and the following chromatographic conditions were optimized.

Optimized Chromatographic Conditions:

LC Conditions

Stationary phase : Phenomenex C₁₈ (50 x 4.6 mm i.d., 5 μ)

Mobile Phase : Methanol: water

Elution mode : Isocratic A: B= 95:5% v/v

Flow rate : 0.7 mL/min

Injection volume: 10 μ L using Auto injector

Oven Temperature: 50°C

MS Conditions

Interface : APCI

Operation mode : SIM /SCAN

Polarity : Positive

Probe temperature : Ambient

CDL Temperature : 250° C

Block Temperature : 200° C

Detector voltage : 1.3kv

Nebulizer Gas flow : 2.5 L/min

Drying gas : 10 L/min

Detection : β -Sitosterol – 397.35

Data station : LC-MS solution data station

The mobile phase was filtered through 0.22 μ membrane and degassed using ultrasonicator. The experiments were carried out at room temperature of about 20°C. Both the standard and sample solutions were injected and the experiment carried out using the above optimized chromatographic conditions and the mass spectrum of β -Sitosterol (Figure 1) and the chromatograms of standard and sample solutions (Figure 2 and 3) were recorded, from which the amount of β -Sitosterol present in the leaf extract was determined.

Estimation of total phenolic content⁸

Preparation of standard solution

Gallic acid is used as the standard which represents the phenolic compound in the plant *Moringa*. 10mg of Gallic acid monohydrate was dissolved in 100mL of methanol to give a concentration of 100 μ g/mL.

Preparation of calibration curve

Aliquots of 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5mL from the above stock solution were taken in 6 different 10mL volumetric flask. To each flask 2.5mL of 1N Folin-Ciocalteu reagent and 2mL of 20% sodium carbonate were added. The mixture was allowed to stand for 15 mins and the volume was made up to mark with water to get a concentration ranging from 2.5-25 μ g/mL. The absorbance of the resulting solutions was measured at 765nm against reagent blank. A standard calibration curve of was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range.

Preparation of sample solution

10mg of extract was dissolved in 10mL of methanol to get 1mg/mL solution. Suitable volume of the above solution was transferred into a 10mL standard flask and color development was carried out as that for standard. Absorbance of the test solution was measured at 765nm against blank. The concentration of total phenol in the test sample was determined by extrapolation from the calibration graph. The total phenol content in the extract was expressed as μ g/mL.

Estimation of total flavonoid content⁹

Preparation of standard solutions

Rutin is used as the standard for estimation of total flavonoids in the prepared extract. 10mg of rutin was dissolved in 10mL of methanol to get 1000 μ g/mL solution.

Preparation of calibration curve

Aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0mL from the above stock solution were taken in 6 different 10mL volumetric flask. To each flask 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate and 2.8 mL of distilled water was added. The reaction mixture was kept aside at room temperature for 30 min and the volume was made up with water. The absorbance of the resulting solutions was measured at 415nm against reagent blank. The calibration curve was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range of 10-100 μ g/mL.

Preparation of test solutions

10mg of extract was dissolved in 10mL of methanol to get 1mg/mL solution. Required volume of the above solution was transferred into a 10mL standard flask and color development was carried out as that for standard. Absorbance of the test solution was measured at 415nm against blank. The concentration of total flavonoids in the test sample was determined by extrapolation from the calibration curve. The total flavonoid content in the extract was expressed as μ g/mL.

Results and Discussion

The quantitative analysis of the hydroalcoholic extract of the leaves of *Moringa oleifera* by LC-MS indicates the presence of 90mg/g of β -sitosterol. The mass spectrum and chromatograms of β -sitosterol are given in the Figure 1-3. The amount of total phenolic and flavonoid compounds present in the hydroalcoholic extract of the leaves of *Moringa oleifera* estimated by Visible-Spectroscopy was found to be 8 μ g/mL and 27 μ g/mL respectively. (Table 1 and 2).

The β -sitosterol isolated from leaves of *Moringa oleifera* is a plant sterol with close chemical resemblance to cholesterol which enables it to block the absorption of cholesterol by competitive inhibition. In the present study, the quantification of β -sitosterol has been done and from the above review it can be concluded that β -sitosterol in *Moringa oleifera* may be responsible for its hypolipidemic and as well as antioxidant properties.

Phenols comprise the largest group of plants secondary metabolite. Phenolic compounds are commonly found in both edible and non-edible plants and they have been reported to have multiple biological effects, including antioxidant property. Total phenol is usually determined in powder crude drugs, extracts and beverages by using the Folin-Ciocalteus method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation a green blue complex formed is measured at 765nm. The total phenol content of a tested material is related to its antioxidant activity.

Flavonoids are water soluble polyphenolic compounds, which are extremely common and wide spread in the plant kingdom as their glycosides. Total flavonol is determined by colorimetric method using aluminium chloride. The principle involved is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum chloride forms acid labile complexes with the ortho-dihydroxyl groups in the ring A- or B- ring of flavonoids.

It is well documented that flavonoids and polyphenols are natural antioxidants. Flavonoids can directly react with superoxide anions and lipid peroxy radical and consequently inhibit or break the chain of lipid peroxidation. This radical scavenging activity of extracts could be related to the antioxidant nature of polyphenols or flavonoids, thus contributing to their electron/hydrogen donating ability. However, further studies are required to isolate the other major flavonoids present in *Moringa oleifera* for its potent antioxidant properties.

Table 1. Estimation of total phenolic compounds

S.No.	Concentration (µg/ml)	Absorbance
1.	2.5	0.021
2.	5	0.042
3.	10	0.062
4.	15	0.082
5.	20	0.103
6.	25	0.123
7.	Test sample (extract)	0.054

Table 2. Estimation of total flavonoid contents

S.No.	Concentration (µg/ml)	Absorbance
1.	10	0.009
2.	20	0.018
3.	40	0.028
4.	60	0.037
5.	80	0.047
6.	100	0.057
7.	Test sample (extract)	0.021

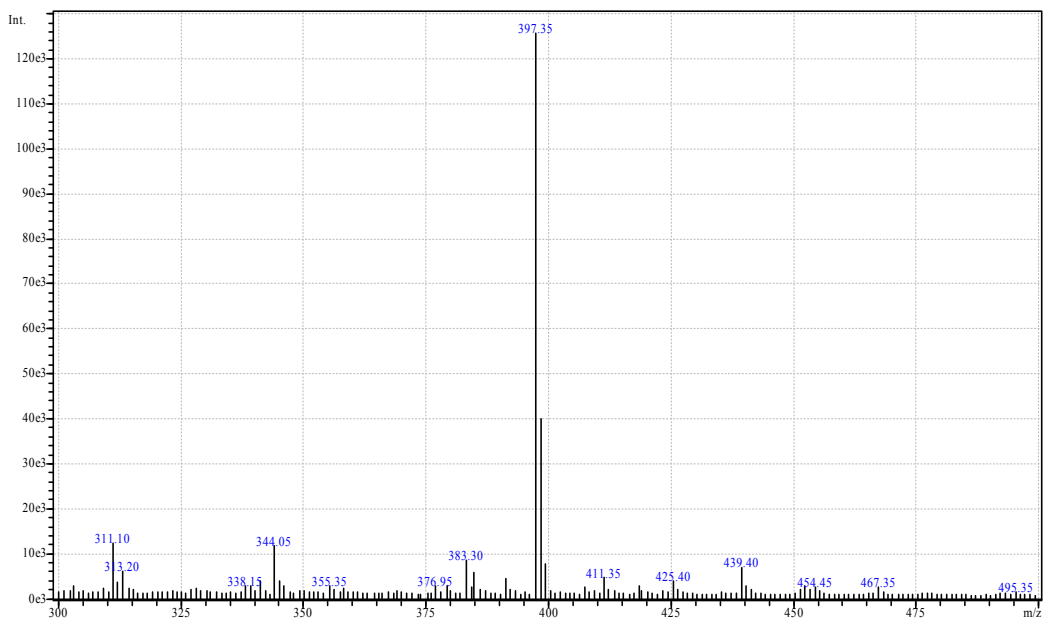
Figure 1. Mass spectrum of β -Sitosterol SCAN mode

Figure 2. Typical chromatogram of standard solution

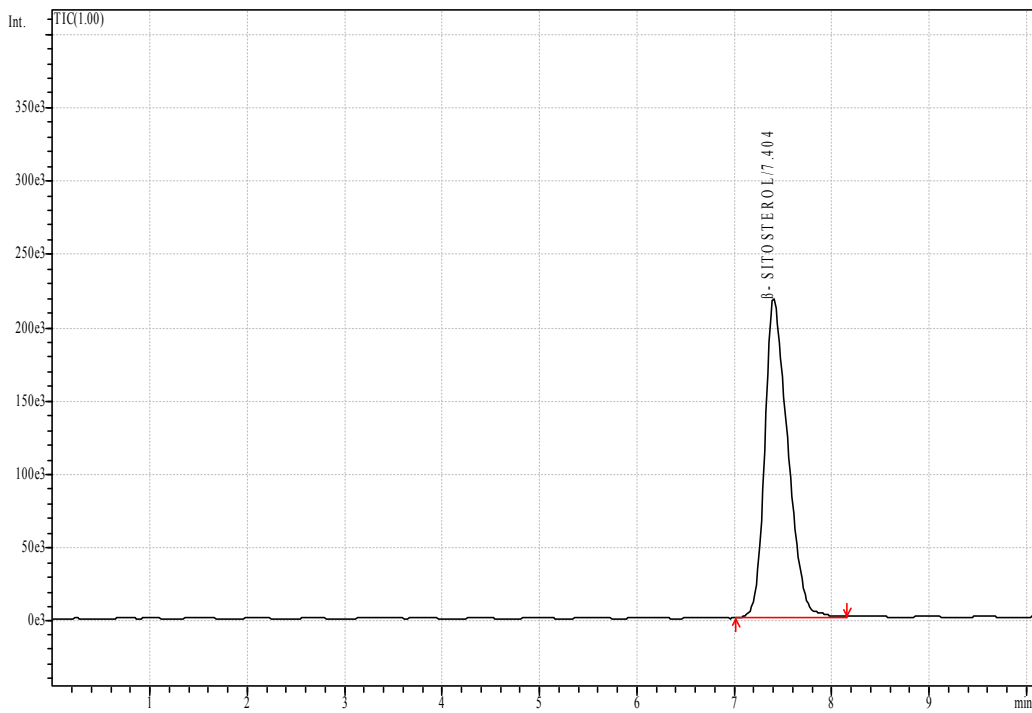
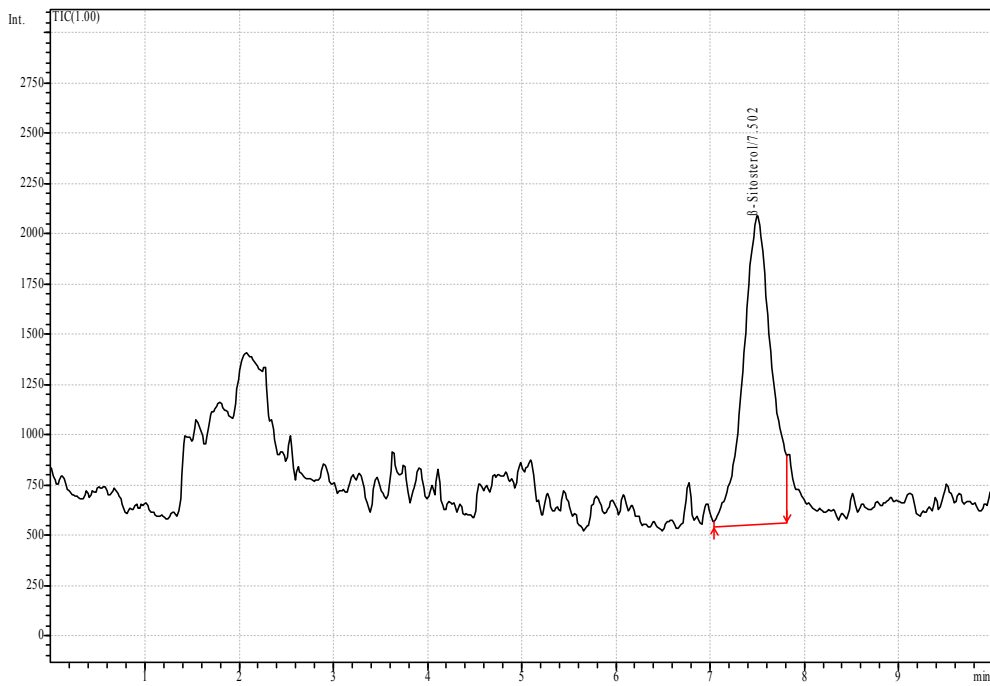


Figure 3. Typical chromatogram of sample solution



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