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Review on different analytical techniques for estimation of active components and vitamins in *Moringa Oliefera*

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Abstract
Objective: This paper discuss the analytical strategies documented in the literature for the estimation of active compounds and vitamins in *Moringa oleifera* leaves by HPLC (High Performance liquid chromatography), HPTLC (High performance thin layer chromatography)
Materials and Procedures: Widely used mobile phases and stationary phases in the literature. In HPTLC - Toluene: ethyl acetate: formic acid, Glass-backed silica gel plates $60F_{254}$-RP18 and $60F_{254}$. In HPLC- Methanol: Glacial acetic acid: water and Methanol: Water, C18 Column.
Conclusion: Validated HPLC, HPTLC Techniques correctly employed for Quantification of vitamins and active components of *Moringa oleifera* leaves. These analytical techniques are cost effective and took less time for the evaluation.

Keywords: *Moringa oleifera*, vitamins, gallic acid, rutin, quercetin, HPTLC, HPLC

Introduction

Drum stick leaves (*Moringa oleifera*) is a rapid expanding, evanescent failure-resistant tree original to the Africa and Asia. It grown in semiarid and tropical regions. Peak-32 – 40toes, Diameter-1.5toes.
Moringa leaves are rich in Vit A, Vit C, Vit B1 (B1Thiamine, Vit B2 (riboflavin), Vit B3 (niacin), Vit B6 and Folate. Magnesium, iron, calcium, phosphorus, and zinc are also abundant [2]. Cooked *moringa* leaves have the same amount of nutrients as raw moringa leaves. As veggies that were inside the day by day eating authority per 100g.
Moringa leaves contain calcium oxalate chargers. Oxalate concentrations might range from 430 mg/100g to 1050 mg/100g [3]. The leaves are dried and ground into a powder, which is then used in curries, mists, and gravies.
It is extensively cultivated for its youthful seed capsules and leaves, used as vegetables and for traditional natural drug. It's also used for water sanctification.
Vitamins are essential for maintaining human health. Vitamin C is an important micronutrient that is found in fruits and vegetables and is required for regular metabolic activities in the human body [1]. *Moringa* leaves have more vitamin A than carrots and more vitamin C than oranges, as well as more calcium, potassium, and iron than milk, bananas, and spinach. Vitamin C is essential for the strength and health of ligaments, tendons, and collagen. Various studies have indicated that a sufficient intake of vitamin C reduces the risk of getting malignancies of the mouth, lung, bone, stomach, cervix, colon, rectum, and prostate [1].
MO's roots, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil are said to provide a variety of biological properties, including gastric ulcer prevention.
The roots, bark, gum, leaf, fruit (pods), flowers, seed, and seed oil of MO are reported to possess various biological activities, including protection against gastric ulcers [4], ant diabetic [5], hypertensive [6] and anti-inflammatory effects [7]. It has also been shown to enhance hepatic and renal functions [8] and therefore the regulation of hormone status [9]. Leaves of this tree protect against oxidative stress [10], inflammation [11], hepatic fibrosis [12], liver damage [13], hypercholesterolemia [14, 15], bacterial activity [16], cancer [17] and liver ulcers. Vitamin C is 10 times much greater than oranges and lemons.
Flavonoids like Quercetin (QT), Rutin (RT), and Phenolic compounds like Gallic acid are found in many plants and herbal treatments (GA). QT is a natural polyphenol found in fruits, vegetables, and juices that has been intensively researched for a range of biological roles. QT, which is chemically aglycone of RT and other glycosides, is a powerful antioxidant and free radical scavenger. Radiotherapy is used to prevent and cure small varicose veins. Cellulite treatment with mesotherapy or intradermotherapy uses this chemical to enhance circulation. It has been used to prepare individuals who have become jaundiced for surgery. GA is a polyphenolic molecule with antioxidant properties that is used as a diuretic, laxative, and liver tonic in addition to treating colds and fevers.

Scientfic Classifiction

- Botanical source: Moringa Oleifera

- Kingdom: Planate
- Division: Magnoliophyta
- Class: Magnoliopsida
- Order: Brassicales
- Family: Moringaceae
- Genus: *Moringa*
- Species: *m. oleifera*

Method for estimation

Chromatographic methods

Various chromatographic techniques like HPLC, HPTLC, GC, UPLC was developed for quantification of vitamins in *Moringa oleifera*. Methods for Quantification of vitamins by HPLC and HPTLC.

Table 1: Determination of Vitamins and Active Compounds in *Moringa olifera* by HPLC

S.no	Name of the plant	Name of the vitamin	Description	R.no
1	*Moringa oleifera*	Vitamin C (Ascorbic acid)	Column: C18 (3.9-mm x 30-cm) Mobile Phase: Glacial acetic acid: methanol: water [1:27:73, v/v/v] Flow Rate: 1ml/min Retention Time: 2.038min	19
2	*Moringa oleifera*	Vitamin B1 (Thiamine)	Column: C18 (3.9-mm x 30-cm) Mobile Phase: Glacial acetic acid: methanol:water [1:27:73, v/v/v] Flow Rate: 1ml/min Retention Time: 11.766min	19
3	*Moringa oleifera*	Vitamin B2 (Riboflavin)	Column: C18 (3.9-mm x 30-cm) Mobile Phase: Glacial acetic acid: methanol: water [1:27:73, v/v/v] Flow Rate: 1ml/min Retention Time: 12.300min	19
4	*Moringa oleifera*	Vitamin B3 (Niacin)	Column: C18 (3.9-mm x 30-cm) Mobile Phase: Glacial acetic acid: methanol: water [1:27:73, v/v/v] Flow Rate: 1ml/min Retention Time: 3.330min	19
5	*Moringa oleifera*	Vitamin B6 (Pyridoxine)	Column: C18 (3.9-mm x 30-cm) MOBILE PHASE: Glacial acetic acid:methanol: water [1:27:73, v/v/v] Flow Rate: 1ml/min Retention Time: 5.786min	19
6	*Moringa oleifera*	Vitamin C (ascorbic acid)	Column: LunaC18(2) (Phenomenex) Mobile Phase: 0.5% NaH2PO4 and acetonitrile (93:7) Flow Rate: 1.2 mL/min Retention Time: 2.060 min	20
7	*Moringa oleifera*	Vitamin B2 (Riboflavin)	Column: C 18 column (5 µm particle size, 250 × 4.6 mm Mobile Phase:0.5%NaH2PO4 and acetonitrile (93:7) Flow Rate: 1ml/min Retention Time: 12.800	20
8	*Moringa oleifera*	Vitamin B9 (Folic acid)	Column: YMC-Pack ODS-AQ column (250×4.6 mm ID× 5 µ) Mobile Phase: 0.1%v/v Trifluoroacetic acid in water: acetonitrile Flow Rate:0.8 ml/min	21
9	*Moringa oleifera*	Kaempferol	Column: Shim-pack CLC –ODS(C18) Column(with 25cm x 4.6m;5 µm Mobile Phase: Aqua-acetic acid: acetonitrile (94:6). Flow Rate: 1ml/min Retention Time: 1.070 min	26
10	*Moringa oleifera*	Quercetin	Column: C18 Symmetry Shield 5 µ 4.60 x 250 mm column Mobile Phase: acetonitrile: water(60:40) Flow Rate: 1ml/min	22
11	*Moringa oleifera*	Rutin	Column: Phenomenex hypersil C18 column (DDS) (Analytical 250 × 4.6C18 column) Mobile Phase: 60% methanol: 1.0% aqueous acetic acid. Flow Rate: 1ml/min	25
12	*Moringa oleifera*	Gallic Acid	Column: Phenomenex hypersil C18 column (DDS) (Analytical 250 × 4.6C18 column) Mobile Phase: 60% methanol: 1.0% aqueous acetic acid. Flow Rate: 1ml/min	25

Table 2: Determination of Active Compounds in Moringa olifera by HPTLC

S.no	Name of the plant	Name of the vitamin	Description	R. No
1	*Moringa oleifera*	Quercetin	Column: Glass-backed silica gel plates $60F_{254}$-RP18 and $60F_{254}$ Mobile Phase: Acetonitrile: water(4:6) Rf Values: 0.23 ± 0.005),	23
2	*Moringa oleifera*	Rutin	Column: Glass-backed silica gel plates $60F_{254}$-RP18 and $60F_{254}$ Mobile Phase: Ethyl acetate: acetone: water: formic acid Rf Values: 0.64±0.02, 0.80±0.03	24
3	*Moringa oleifera*	Naringenin	Column: Glass backed silica gel plate's $60F_{254}$-RP18 and $60F_{254}$. Mobilephase: Toluene: ethylcetate: formic acid, 6:4:0.8 Rf Values: (0.56 ± 0.009	23
4	*Moringa oleifera*	Gallic Acid	Column: Glass backed silica gel plate's $60F_{254}$-RP18 and $60F_{254}$. Mobile Phase: Toluene: ethylcetate: formic acid(6:4:0.8) Rf Values: (0.28 ± 0.006).	23
5	*Moringa oleifera*	Chlorogenic Acid	Column: Glass Backed Plates (10cm × 20 m)coated silica gel 60 F_{254} Mobile Phase: Ethyl acetate: acetone: water: formic acid (6:3:2:2(v/v)) Rf Values: (0.033 ± 0.005%)	24
6	*Moringa oleifera*	Gallic Acid	COLUMN: Glass Backed Plate (10 cm × 20 cm) coated silica gel 60 F_{254} Mobile Phase: Ethyl acetate: acetone: water: formic acid (6:3:2:2(v/v)) Rf Values: (0.031 ± 0.005%),	24

Table 3: Parameters of HPLC and HPTLC

Parameters	HPLC	HPTLC
Type	Reverse phase Chromatography	Straight phase Chromatography
Stationary phase	Liquid	Solid
Separation	Partition	Adsorption
Analysis	On-line	Off-line
Resolution	Very high	Moderate –High
Chromatography System	Closed	Open
Separating Medium	Tubular column	Plate
High temperature/pressure	High pressure	None
Analysis in Parallel	Only 1 at a time	Up to 100 samples at a time
Fractions seen as	Broad peaks	Wide peaks
Time per Sample	2-60min	1-30min
Data obtained	Limited to very high	High to very high
Sensitivity	High to ultra	High moderate to ultra-high
Fluorescence data	Possible, optional	Possible, inbuilt
Detectors	PDA, UV, Fluorescence	UV-visible, Bioluminescence
Chromatogram documentation	No	At 254 and 366 & visible
Analysis cost	Very high	Low
Equipment Maintenance	Very high	Low
Skills Required	Very high	High

Conclusion

Finally, it was determined that this paper reflects the estimation of numerous vitamins and active components found in *moringa oleifera*. In HPTLC Glass-backed silica gel plate's 60F254-RP18, Toluene: ethyl acetate and formic acid and the C18 Column is employed by the majority of researchers in HPLC-Methanol: Glacial acetic acid: water, and Methanol: Water because of its sensitivity, repeatability, and efficiency in separation. Any researcher who reads this material in the future will benefit from the examination of nutrients and active components.

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