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**Saifur Rehman Khattak**Central Drug Laboratory,  
Karachi, Pakistan**Amjad Hussain**Department of Eastern Medicine,  
Faculty of Pharmacy & Health  
Sciences, University of  
Balochistan, Quetta, Pakistan**Aadil Ameer Ali**Department of physiotherapy,  
Faculty of Pharmacy & Health  
Sciences, University of  
Balochistan, Quetta, Pakistan**Taufiq Ahmad**Department of Eastern Medicine,  
Faculty of Pharmacy & Health  
Sciences, University of  
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Government College University,  
Faisalabad, Pakistan**Muhammad Ishaque MR**Department of Eastern Medicine,  
Faculty of Pharmacy & Health  
Sciences, University of  
Balochistan, Quetta, Pakistan**Wahid-ullah**Wilson pharmaceuticals,  
Islamabad, Pakistan**Corresponding Author:****Amjad Hussain**Department of Eastern Medicine,  
Faculty of Pharmacy & Health  
Sciences, University of  
Balochistan, Quetta, Pakistan

## Comparative studies of leaves and bark of *Moringa oleifera* originated from Khyber Pakhtoon Khwa and Punjab, Pakistan

**Saifur Rehman Khattak, Amjad Hussain, Aadil Ameer Ali, Taufiq Ahmad, Sultan Ayaz, Muhammad Akram, Muhammad Ishaque MR and Wahid-ullah**

### Abstract

*Moringa oleifera* has been used extensively for its nutritious and therapeutic value. Numerous parts of the plant such as flower, root, leaves, seeds and bark are used for these purposes. In Pakistan the use of moringa plants is not very common, however, due to some scientific work and subsequent promotion by some workers its use is gaining importance day by day. The current study has been undertaken to scientifically explore some aspects of the plant particularly leaves and bark by using chromatographic techniques such as TLC and HPLC. The Thin layer chromatography (TLC) separates only two vitamins (vitamins B2 and B6) with the help of methanol and Ammonia solution (100:1.5 v/v) as a standard mobile phase whereas the HPLC have cause separation of vitamin B1 (Thiamin), B2(Riboflavin), B3(Niacin), B6(pyridoxine) and Ascorbic acid (vitamin C).

**Keywords:** *Moringa oleifera*, FTIR, UV spectroscopy, TLC, water soluble vitamins

### Introduction

History shows that medicinal plants are used from antique time. In Nagpur, around 5000 years ago evidence of herbal treatment has been found. A Chinese writer Emperor Shen Nung wrote a book in 2500 BC which contain 365 medicinal plants. Most of those plants like ginseng, ephedra, podophyllum, jimson weed, camphor, *Theae folium*, and cinnamon bark are also used nowadays. The most important and oldest book of Egypt "Ebers Papyrus" written in 1550 BC, which composed of 700 plant species. Hippocrates the father of medicine has classified 300 medicinal plant on the base of biological/ pharmacological activities like antipyretic, antiarthritic, styptic, antimalarial, demulcent, anthelmintic, diuretics, sedative, narcotics, antiepileptic, anticancer, and astringent [1].

Worldwide therapeutics plants are used for healing various maladies. It is estimated by WHO that about 80% of people used medicinal plants for their primary health care in less developed countries [2].

Medicinal Plants are the most common source used as medicine to maintain good health. At ancient time medicinal plants were used in the crude form. Now a day's herbal medicine are used for various ailments regarding their active ingredients [3, 4].

Various Medicinal plants such as *Emilia coccinea*, *Cleome nutidosperma*, *Tridax procumbens*, *Richardia bransitensis*, *Spigelia anthelmia*, *Scopania dulcis*, *Stachytarpheta cayennensis*, *Sida acuta*, *Euphorbia heterophylla* and *Physalis angulata* were investigated for phytochemical analysis that contain different chemical constituents such as alkaloids, minerals, tannins, vitamins, saponins, flavonoids, isoflavonoids, steroids, terpenoid, terpenes, isoterpenes, cardiac glycoside, phenols, polyphenols and phlobatannin which possesses various pharmacological activities and are used for prevention of different diseases [5, 6].

South Africa has a vast diversity for medicinal plants. The flora of Southern Africa contain about 30,000 species of higher plants about which 9000 species belongs to the Cape Floral Kingdom. 3000 species are used medicinally, in which 350 plants species are most commonly used for therapeutic purpose [7].

According to WHO about 70% of the world population uses therapeutic agent derived from medicinal plants by Hakims to cure many diseases. Research evidence shows that India has several system of medicine, such as Ayurveda and Unani, which has mainly dependent on medicinal plants. WHO conducted a survey that shows 60%, 65%, 75%, 85%, 90% patients in Pakistan Srilanka, Nepal, Myanmar and Bangladesh respectively are treated by traditional

practioners. In India 80% of population of rural area uses herbal preparations for primary health care [8, 6].

In Pakistan various herbal remedies are used for different ailments. In rural areas, more than 80% of population of Pakistan is dependent on herbal remedies for primary health care. Now day's the use of herbal remedies is diverting towards the urban areas. About 6000 plant species have been recorded in Pakistan. Out of these 6000 plant species 600 plants species have been recognized as a medicinal plants [9]. Nutrients are the substances that are required for regulation of normal chemical processes occurring in living organism. Ethnobotanists, nutritionists, clinicians and chemists are interested to know the nutritional plants which has best nutritional value and used for nutritional as well as therapeutics purposes. Due to its extensive nutritional value it is used as a nutritional supplement as well as for various ailments [10].

*Moringa Oleifera* (MO) family Moringaceae, genus Moringa is commonly known as Moringa or Sohanjna or Drumstick or Benzoi tree or Miracle tree or Horseradish tree, Sohanjna, Tree of life, Kelor, Mother's best friend and Malunggay [10, 11]. Plants produce primary and secondary metabolites such as flavonoids, isoflavonoids, terpenes, terpinoids, glycosides, alkaloids (moringinine), saponins, phytosterols ( $\beta$ -sitosterol), quercetin, Chlorogenic acid, Aurantiamide acetate, phenolic compound, carbohydrate, protein, benzyl isothiocyanate, niazimicin, pterygosperrin, benzyl glucosinolate,  $\beta$ - carotene, zeatin, kaempferol and tannin. Research shows that *Moringa Oleifera* contains all these metabolites in their leaves, bark, seeds, flowers and roots which are therapeutically useful as antipyretic, antitumor, antioxidant, cardiac stimulants, antidiabetic, antiepileptic, antibacterial, anti-inflammatory, antifungal, hepatoprotective, antispasmodic, antihypertensive, antiulcer, antidiabetic, diuretic and cholesterol lowering effects [10-14].

*Moringa Oleifera* contains micro minerals as well as macro minerals. These minerals are Calcium, Magnesium, Potassium, Sodium, Iron, Zinc, Phosphorus, Manganese, Copper, Sulphur and Selenium which prevent various diseases and promotion of good health [15].

*Moringa Oleifera* is cultivated for commercial use in different countries. Pakistan also cultivated it and use of this plant is increases day by day. The current study shows comparative study of leaves and bark of MO through chromatographic technique (high performance liquid chromatography).

## Material and Methods

### Plant Material

The leaves and bark of *Moringa oleifera* were collected from two provinces of Pakistan [Punjab (Tounsa), and KPK (Kohat)]. The plant sample were identified by Dr. Shafi Muhammad Chairperson Department of Pharmacognosy University of Balochistan Quetta, Voucher specimen No. MA.400 was deposited in the department of Pharmacognosy.

### Methods

#### Preparation of Plant Material

*Moringa oleifera* is grown in Tounsa (Punjab) and Kohat (KPK). Leaves and bark were collected in the month of April to avoid seasonal effects and perform the following procedures.

#### Grinding of plant Leaves and Bark

Fresh Leaves and bark of MO were crushed after drying. The dried bark and leaves were broken down and crushed into

very small pieces with the help of cleaned kitchen grinder. After that, the both sample were powdered with the help of mortar and pestle [16, 17].

### Extraction

1g of powder sample (leaves and barks) were soaked in 100 ml of water for 24hrs. Then the solution was filter through filter paper (0.45 $\mu$ ). The filtration of leaves and bark were kept in air tight glass container and an aluminium foil was surrounded to prevent the degradation of substances (multivitamins) and used within 24hrs [18, 19].

### Chromatographic Evaluation

Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) were used in the current study. Extracted samples were applied on TLC plate and ascends chromatography by using a solvent of methanol and ammonia (100:1.5 v/v). Reference standards of vitamins were dissolved in methanol and applied to the TLC plates along with samples and ascend upto the solvent front (6cm). The plate was dried under air and viewed the spots with the help of ultraviolet light at 254 and 366 nm. The spots were circle with the help of pencil [20, 21]. The chromatographic technique (HPLC) analysis was performed as below:

### Chromatographic Conditions

**Mobile Phase:** A solution of glacial acetic acid (1ml), methanol (27ml) and water (73ml) [1:27:73, v/v/v] comprising of 140mg of sodium hexane-1-sulfonate per 100ml.

**Diluent:** Water (94ml), acetonitrile (5ml) and glacial acetic acid (1ml).

**Standard Solution:** put 20mg of reference standard of Ascorbic acid, Pyridoxine HCl, Nicotinamide, Thiamine HCl and Riboflavin 5-phosphate respectively to 200ml graduated flask and diluted with the addition of 180ml of Diluent solution. Submerged the flask in a hot water bath for 10 minutes at a control temperature of 65° – 70° with continuous shaking through a vortex mixer, to dissolve all the solid ingredients. After completion of dissolution of the solid material dip the flask quickly in a cold water bath at room temperature for 10min.

**Sample Solution:** 15ml of extracted sample of leaves and bark along with 15ml of diluent was added to a test tube and mixed well for 30 seconds to completely dissolve the extract of leaves and bark with the help of vortex mixer. Test tube was immersed in a hot water bath for 5 mints at a control temperature of 65°- 70° and mixed well by using vortex mixer for 30second. Again the test tube was heated in a hot water bath for another 5 mints and mixed for 30 second with the help of vortex mixer. Filtered the solution with the help of filter paper and cooled it at room temperature. This clear filtrated solution was used within 3hour [22, 23].

S.no	Description	Conditions
1	HPLC Column	3.9-mm x 30-cm
2	Detector	UV 280nm
3	Injection volume	10 $\mu$ l
4	Flow rate	1ml/minute

### HPLC System suitability

**Sample:** Standard solution

**Suitability requirements****Relative standard deviation (RSD):** NMT 3.0%

**Analysis:** 20 $\mu$  of standard solution and extracted solution of leaves and bark was injected respectively to the chromatograph. The peak areas for water soluble vitamins such as Vit C, Vit B1, Vit B2, Vit B3 and Vit B6 was measured. The following formula is used for the calculation of the percentage amount of these vitamins [24].

$$C_u = \frac{r_u \times C_s \times 100}{r_s}$$

$r_u$  = peak area of corresponding vitamin from the specimen mixture

$r_s$  = peak area of corresponding vitamin from the standard mixture

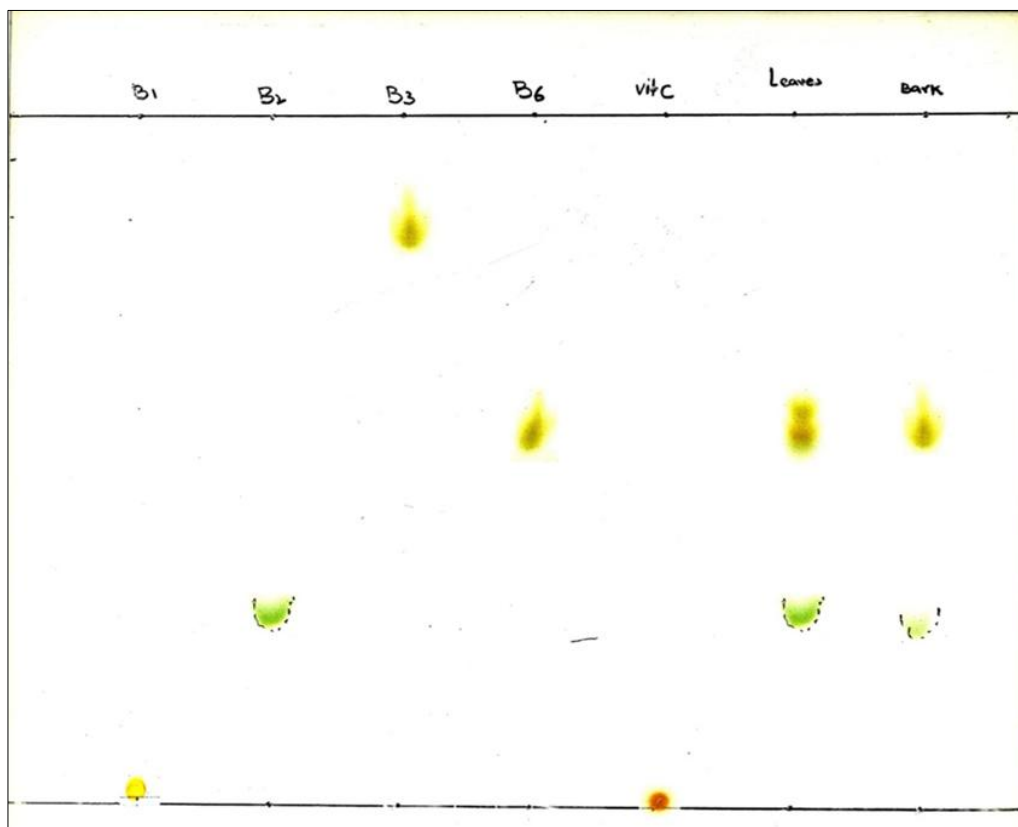
$C_s$  = concentration of relevant reference standard in the standard solution

$C_u$  = concentration of corresponding vitamin in the specimen mixture

**Results**

**1. Identification technique of water soluble vitamins (B1, B2, B3, B6 & Vit C) in leaves and bark extract of MO by using TLC methods**

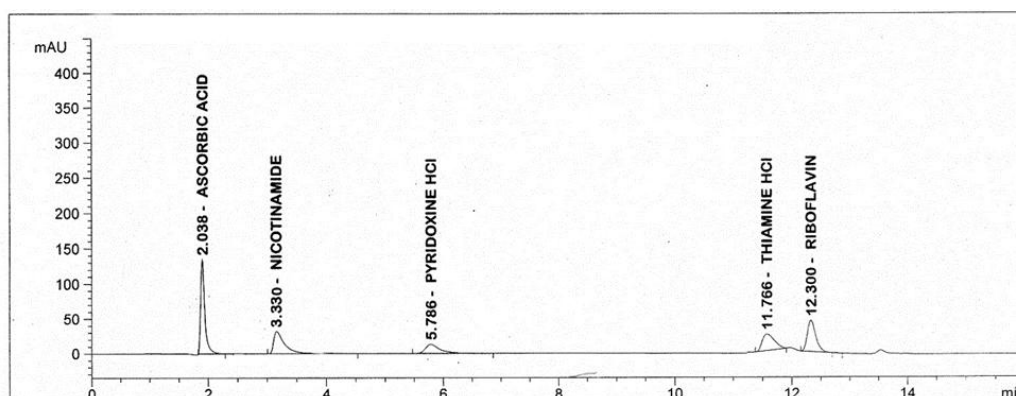
TLC has isolated only vitamin B2 and B6 (Figure 1).



**Fig 1:** Thin layer chromatogram of standard vitamins and water extracted samples (Leaves and Bark) of MO.

**2. Identification and quantification of vitamin C and B-complex vitamins in bark and leaves of MORINGA OLEIFERA by employing HPLC methods:** HPLC is the effective and reliable technique used for identification and quantification of vit C, Thiamine, Nicotinamide,

Riboflavin and Vitamin B6 from leaves and bark extract of MO. Both the leaves and bark chromatogram (Figure 2 and 3 respectively) are compared with the reference standard chromatogram (Figure 4).



**Fig 2:** Typical HPLC chromatogram of water extract of Horseradish tree leaves.



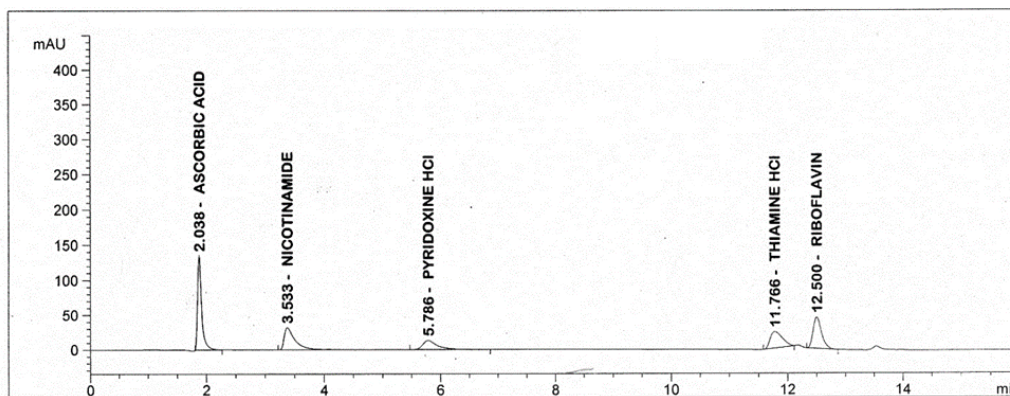


Fig 3: HPLC chromatogram of water extract of Drumstick tree bark.

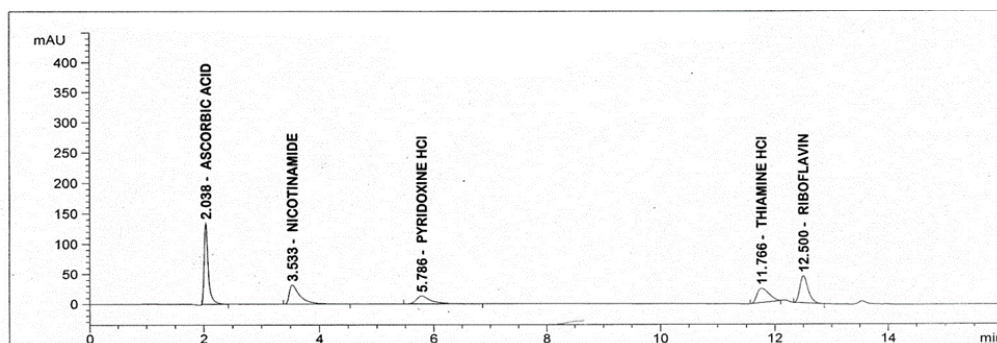


Fig 4: HPLC chromatogram of water solution of Vitamin C and Vit.B1, B2, B3 and B6.

Leaves and bark sample of ben oil tree were collected from two provinces of Pakistan (Punjab and Khyber pakhtoon khwa (KPK). Plant material was soaked in water for 24 hours at room temperature. HPLC was performed to analyzed water soluble vitamins. The method of HPLC was validated in respect of four parameters such as accuracy, linearity, precision and specificity. Analysis results have been tabulated in table no.1.

**Table 1:** Microgram per gram ( $\mu\text{g/g}$ ) powder of each sample were macerated in water for 24 hours. Assay of the samples is tabulated below.

Province	$\mu\text{g/g}$ Leaves powder Extracted in 100ml water					$\mu\text{g/g}$ Bark powder Extracted in 100ml water				
	As. Acid	NA	RF	PD	TA	As. Acid	NA	RF	PD	TA
Punjab	2130	62	306	1910	425	2740	13	144	880	173
KPK	4780	51	322	1770	379	2510	16	182	1120	156

## Conclusion

*Moringa oleifera* contains abundant amount of water soluble vitamins [14, 25-28]. The relevant study demonstrated the phytochemical analysis of *Moringa Oleifera*. Results of this study concluded that thin layer chromatography has isolated only vitamin B2 and vitamin B6. The results of HPLC chromatogram shows that high performance liquid chromatography (HPLC) has effective method for identification and quantification of extracted water soluble vitamins in leaves and bark of *Moringa oleifera*. Both the retention times (Rt) of reference standards and extracted samples of leaves and bark for vitamins were compared with each other and the outcomes are mention in the result portion. All these vitamins has been quantified by applying the USP-41 HPLC method. Before quantification, the method has been properly validated regarding precision, accuracy, specificity, and linearity.

The results of this study shows that the plant contains thiamine, pyridoxine, ascorbic acid, nicotinamide and riboflavin in various concentration. It is concluded that Punjab sample contains higher amount of vitamins as compared to KPK Pakistan. This variation may be due to fertility of the land. The results also described that the leaves extract contains higher amount of vitamins as compared to the bark extract. This observation in consistent with other published research [29].

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