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CHARACTERIZATION OF SOME ASPECTS OF *MORINGA OLEIFERA* LEAVES AND BARK EMPLOYING SPECTROSCOPIC AND CHROMATOGRAPHIC TECHNIQUES

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ABSTRACT

Moringa plant has been used widely for its nutritional and therapeutic value. Different parts of the plant such as leaves, bark, stem roots, seeds and flowers 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 microscopic, spectroscopic and chromatographic techniques. The microscopic studies have shown that leaves consist of epidermal, xylem, phloem and lower epidermal layers composed of numerous cells whereas the bark consist of the cork cells, cork cambium and secondary cortex layers respectively. Distinct FTIR spectra have been obtained on the powdered samples of the plant material. The UV spectra of the plant material in various solvents such as 1N HCl, 1N NaOH, Methanol and Ethanol have shown varying absorption maxima indicating the extraction of different constituents in these solvents. The Thin layer

chromatography (TLC) studies under standard conditions with mobile phase of methanol: Ammonia (100:1.5, v/v) have shown the separation of vitamin B2 and B6 only whereas the HPLC have cause separation of vitamin B1, B2, B3, B6 and vitamin C. Drying and extraction processes have impacted the contents of water soluble vitamins in the plant material.

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

INTRODUCTION

Traditional medications are used for the treatment of different diseases in health care system worldwide [1]. Major portion of these medicines belong to plant origin. Since these medicines have less side effects and also provides complementary remedies for various threatening diseases [2]. Herbal extracts contain different chemical constituents which are biologically dynamic and are used for different purposes like supplement, aromas therapy, cosmetics and dyes etc [3].It is estimated that approximately 15 million of adults took prescription alternative remedies in 1997 in USA, whereas, more than 70% population of the developing world depend on these medicines [4, 5].

Due to wide use of these medicines their manufacturing industry is developing at rapid pace [6]. Some estimation shows that the global marketing of medicinal plants may reach $5 trillion by 2050 [7]. One study shows that China has highest rank in the export of medicinal plants while India, Germany and USA also have massive exports of medicinal plants [8].

In Pakistan various herbal remedies are used for different diseases [9]. More than 80% Pakistani population is dependent on alternative remedies for primary healthcare in the rural areas, which is diverting rapidly towards the urban areas. In Pakistan about 6000 plant species have been recognized and Ethno-medicinally, about 600 plants species have been recorded out of these 6000 plants [10].

Moringa oleifera is one of the most important plants used extensively for nutritional and therapeutic purposes. It belongs to the genus Moringa family *Moringaceae*. It is commonly known as Miracle tree or Sohanjna or Moringa or Drumstick tree or Horseradish tree or Benzoil tree [11].

Different researchers have shown the entire parts of moringa tree like seeds, leaves, roots, bark and flowers therapeutically useful as antitumor, cardiac stimulants, antiepileptic, anti-

inflammatory, antipyretic, antispasmodic, antiulcer, diuretic, cholesterol lowering, antihypertensive, hepatoprotective, antioxidant, antidiabetic, antifungal, antibacterial [12].

The plant containsvarious important elements like Ca, Mg, Zn, K, Fe, Na, Cu, Sulphar, Phosphorus, Manganese, selenium etc. It also contain natural hormones like auxin, cytokinin and zeatin in excess amounts. It is also rich in carbohydrates, proteins and vitamins such as vitamin A, vitamin B-complex, vitamin C and vitamin E [13, 14, 15, 16].

Moringa plant has been used extensively from very old days hence, the Romans, Egyptians and the Greeks extracted oil from the seeds and used it in perfume and skin lotionsfor different purposes [17, 18]. The use of moringa tree in Pakistan is also on rise day by day. No systematic work could be found in the literature on the various aspects of the plant originated from different parts of the country. The current study is an effort to characterize the leaves and bark of the plant through microscopic, spectroscopic and chromatographic techniques and also to analyze for water soluble vitamin contents under various drying and extraction methods.

MATERIAL AND METHOD

Plant Material

Moringa oleifera leaves and bark were collected from all provinces ofPakistan(Punjab (Tounsa), Sindh (Karachi), KPK (Kohat) and Balochistan (Hub) Water souluble vitamins determination. For other studies plant from KPK providence was selected. The plant was identified, sample Voucher specimen No.MA.396 was deposited in the department of Pharmacognosy university of Balochistan Quetta.

Methods

Preparation of Plant Material

Leaves and bark of moringa plant cultivated in Karachi (Sindh), D.G Khan (Punjab), Kohat (KPK) and Hub (Balochistan) were collected at the same time to avoid any effect of season and subjected to the following process for detail study.

Grinding of plant material

Fresh leaves and bark were dried under shade before crushing. The dried bark was broken into small pieces and then crushed in cleaned kitchen grinder whereas; dried leaves of the plant were powdered with the help of pestle and mortar [19].

Extraction

Exactly weighed 1g quantities of powder samples of leaves and barks of moringa plant were soaked in 100ml of different

solvents like 1N HCl, 1N NaOH, Methanol, Ethanol and water for 24 hours and then filtered through 0.45µ filter paper. The filtration was stored in air tight glass container in refrigerator and used within 24hrs [20, 21].

The powder samples (1g) were also extracted with 100ml water at 30 ± 2°C, 40 ± 2°C and 60 ± 2°Cfor 5 hours with gentle shaking in water bath before filtration and HPLC analysis to study effect of temperature on the concentration of multivitamins extracted [22, 23].

Spectroscopic Evaluation

FTIR and UV-visible Spectroscopy were used in this study. For FTIR spectroscopy powder samples of the leaves and barks were placed directly on the pre-acetone cleaned sample probe of the calibrated FTIR spectrophotometer and spectra obtained, respectively. For UV-visible spectroscopy leaves and barks samples were extracted first in various solvents. The extracts were filtered through 0.45µ filter paper and then subjected to UV-visible spectroscopy [24].

Chromatographic Evaluation

The study involved thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). For TLC an appropriate volume of the sample extract was applied to silica gel 254 pre-coated plates and subjected to ascending chromatography using methanol : ammonia (100:1.5, v/v) as the developing solvent[25]. Reference standards of the vitamins dissolved in methanol were also applied to the TLC plates along with samples. The solvent was allowed to ascend the plate upto a distance of 6cm. The plate was then air dried and viewed under ultraviolet light at 254 and 366 nm to locate the spots. The spots were carefully marked with pencil and then covered with scotch tape for protection. The HPLC analysis was performed as under:

Chromatographic Conditions

Mobile Phase: A mixture of methanol, glacial acetic acid and water (27:1:73, v/v/v) containing 140mg of sodium 1-hexanesulfonate per 100ml.

Diluent: Acetonitrile, glacial acetic acid and water (5:1:94)

Standard Solution: Transferred 20mg Ascorbic acid RS, 20mg Pyridoxine HCl RS, 20mg Nicotinamide RS, 20mg of Riboflavin 5-phospate RS, and 20mg of Thiamine HCl RS, to 200ml volumetric flask and added 180ml of Diluent. Immersed the flask in a hot water bath maintained at 65° – 70° for 10min with regular shaking or using a vortex mixer,

until all the solid materials were dissolved. Chilled rapidly in a cold water bath for 10min to room temperature, and diluted with Diluent to volume.

Sample Solution: Transferred 15ml extract of moringa leaves or bark to a 50ml centrifuge tube. Added 15ml of diluent and mixed using a vortex mixer for 30 s to completely dissolve the sample. Immersed the centrifuge tube in a hot water bath maintained at 65^{o}- 70^{o}, heated for 5min and mixed using a vortex mixer for 30s. Returned the tube to the hot water bath, heated for another 5min and mixed on a vortex mixer for 30s. Filtered a portion of the solution, then cooled to room temperature and used the clear filtrate. Used the filtrate within 3h of filtration.

Detector: UV 280nm

Column: 3.9-mm x 30-cm; packing L1

Flow rate: 1ml /min

Injection volume: 10µl

System suitability:

Sample: Standard solution

Suitability requirements:

Relative standard deviation: NMT 3.0%

Analysis: Injected 20µ each of the standard preparation and sample preparation to the chromatograph and then measured the peak areas for ascorbic acid, Nicotinamide, pyridoxine, riboflavin and thiamine. Calculated the percentage of the amount of ascorbic acid, Nicotinamide, thiamine, riboflavin and pyridoxine [26-28]using the following formula.

$$Cu = \frac{r_u \text{x } Cs \text{ x } 100}{R_s}$$

r_u = peak area of corresponding vitamin from the sample solution

r_s = peak area of corresponding vitamin from the standard solution

C_s = concentration of relevant RS in the standard solution

C_u = concentration of corresponding vitamin in the sample solution

RESULT AND DICUSSION

1. **Identification of the leaves and bark employing microscopic and spectroscopic methods**

The microscopy results have shown that leaves of moringa plant consist of upper epidermal, xylem, phloem and lower epidermal layers composed of various cells and starch granules. The bark is composed of three distinct layers such as Cork, Cork Cambium and Secondary Cortex with Calcium oxalate crystals, Starch granules and oil globules.

The FTIR spectrophotometry and UV-visible spectrophotometry have shown distinct spectra for leaves and bark samples (Figure 1-4).

2. **Identification and quantification of chromatographic methods of water**

soluble vitamins in leaves and bark employing chromatographic methods

Thin layer chromatography (TLC) has separated two vitamins i.e. vitamin B2 and B6 (Figure 5).The high performance liquid chromatography (HPLC) studies have shown to be used the effectively for identification and quantification of water soluble vitamins (Ascorbic Acid, Thiamine, Riboflavin, Nicotinamide and Pyridoxine) in moringa leaves and bark extracts (Figure 6).

The assay of water soluble vitamins in water extracts, produced by macerating the plant material for 24hrs without heating of moringa leaves and bark samples collected from the four provinces, was performed by HPLC method validated in respect of linearity, specificity, precision and accuracy. The results of assay have been presented in Table 1.

3. **Effect of drying and extraction methods on the assay of water soluble vitamins in moringa leaves and bark.**

To study the effect of drying and extraction methods on the assay of multivitamins, the plant material were dried under sun and in shade and then extracted in water for five hours at 30 ^{o}C, 40 ^{o}C and 60 ^{o}Cwith gentle shaking. The HPLC assay results have been shown in Table 2.

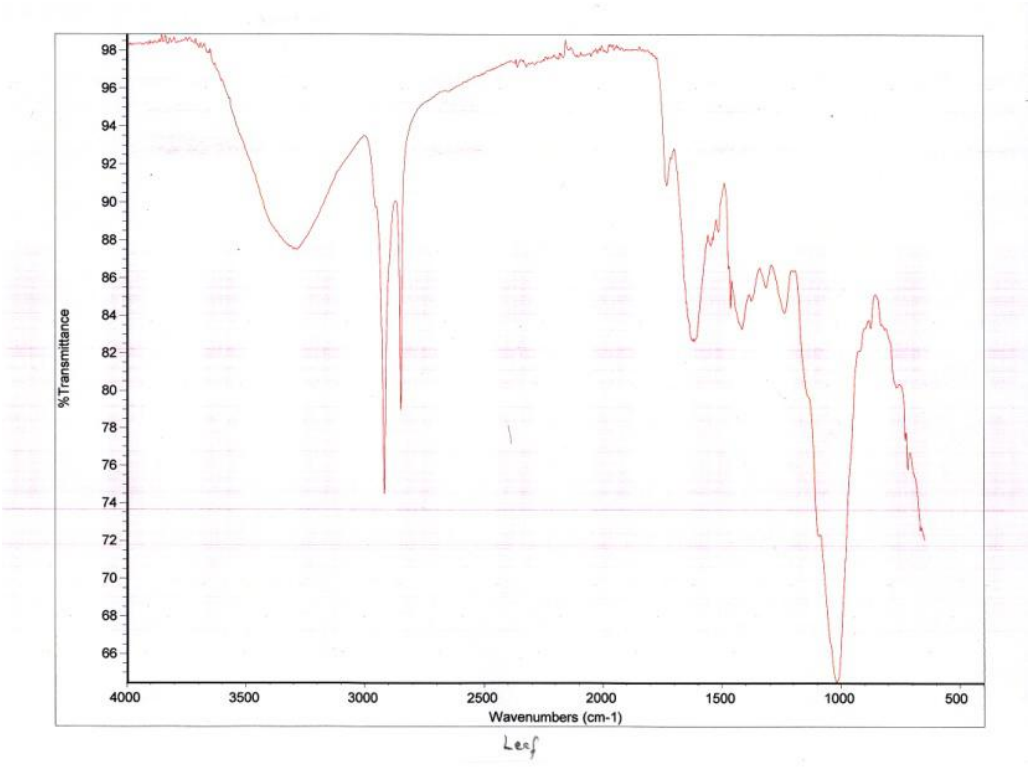

Figure 1: FTIR spectrum of *Moringa oleifera* leaves powder

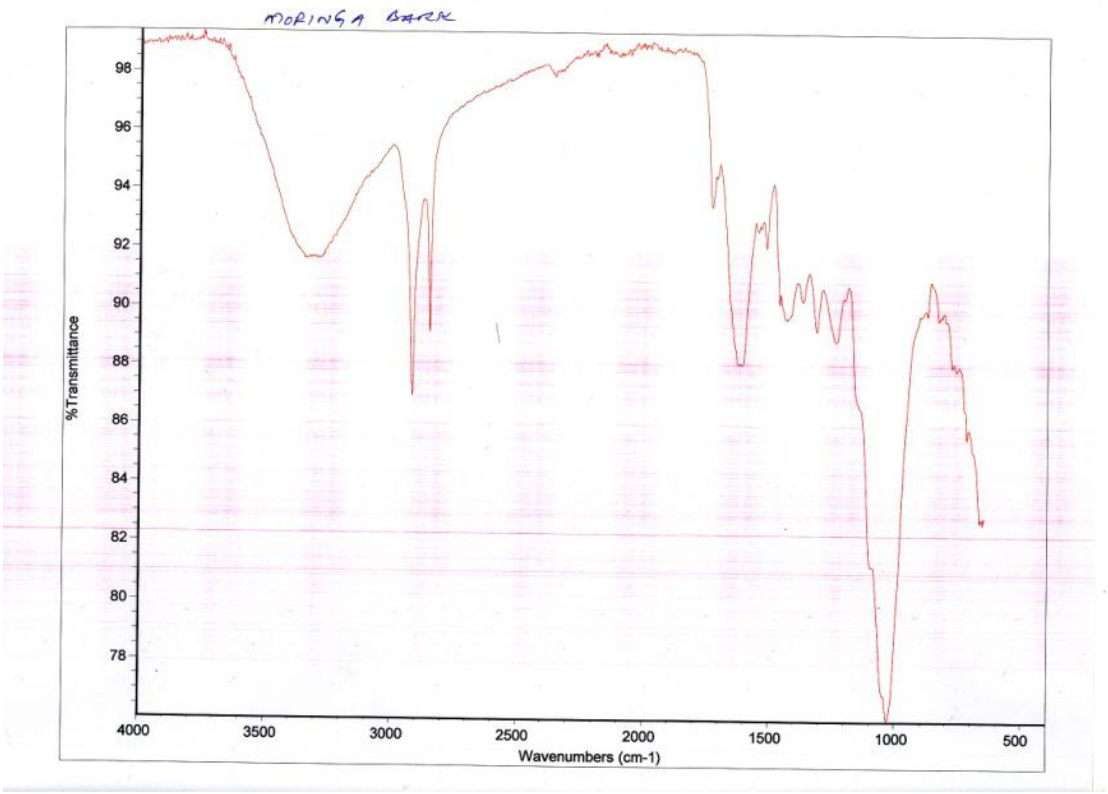

Figure 2: FTIR spectrum of *Moringa oleifera* bark powder

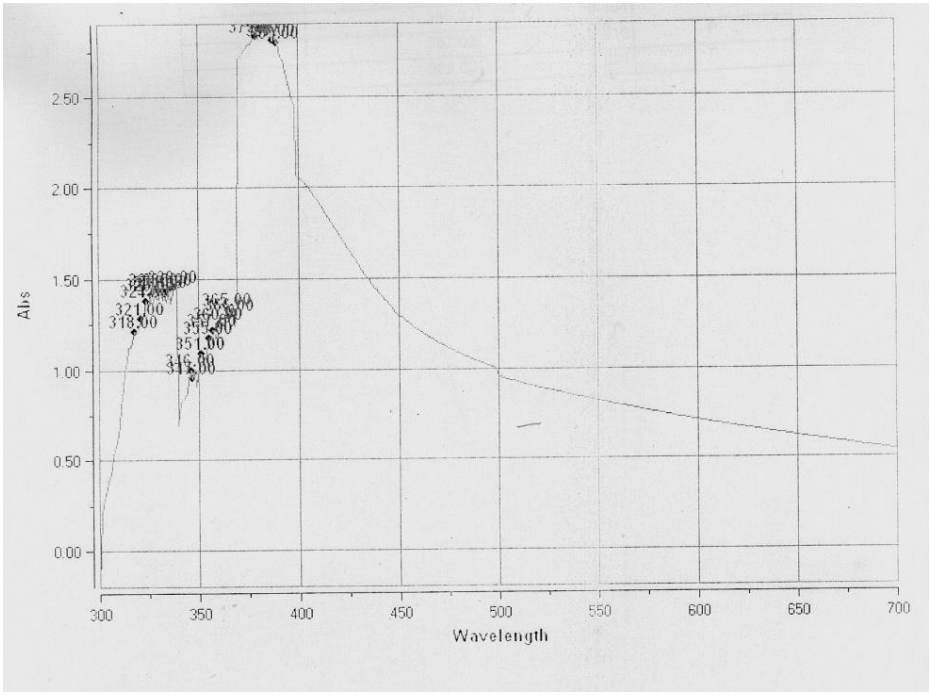

Figure 3: UV spectrum of water extract of Moringa leaves

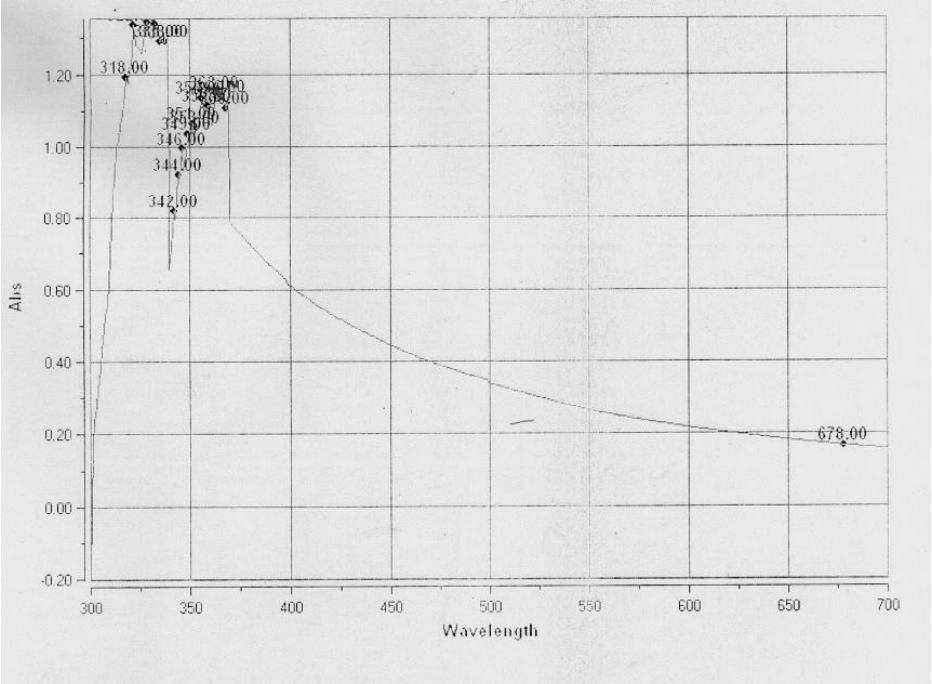

Figure 4: UV spectrum of water extract of Moringa bark

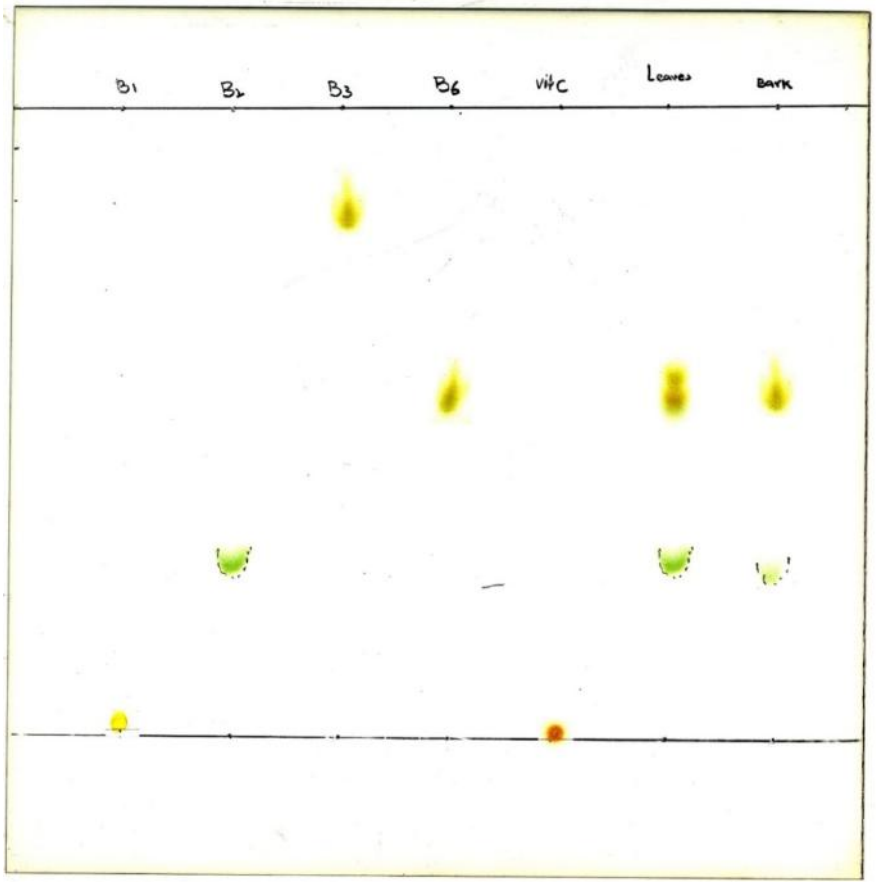

Figure 5:Thin layer chromatogram of *Moringa oleifera* leaves and bark water extracts and the vitamins standards

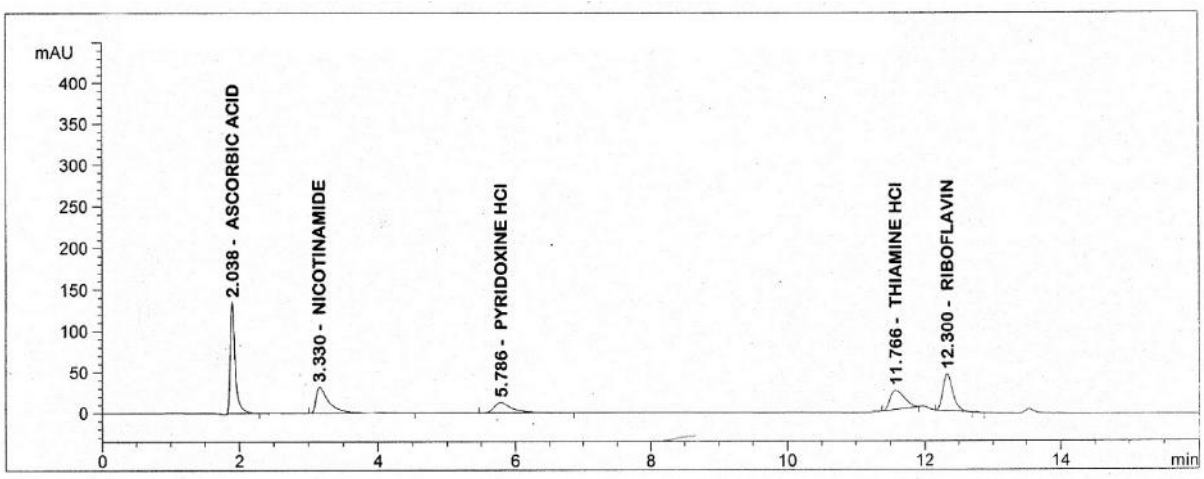

Figure 6: Typical HPLC chromatogram of water extract of *Moringa oleifera* leaves / bark extract

Table 1: Assay of µg/g powder of water soluble vitamins in water extracts (24 hours maceration without heating) of undried moringa leaves and barks collected from four provinces of the country

Province	Leaves Powder (µg/g powder extracted in 100ml water)					Bark Powder (µg/g powder extracted in 100ml water)				
	As. Acid	Nico	Ribo	Pyri	Thia	As. Acid	Nico	Ribo	Pyri	Thia
Punjab	2130	62	306	1910	425	2740	13	144	880	173
KPK	4780	51	322	1770	379	2510	16	182	1120	156
Sindh	4290	37	218	1250	483	2840	32	189	940	117
Balochistan	4020	28	241	0.98	413	3090	27	160	845	89

Table 2: Assay of samples of moringa leaves and bark dried and extracted through different methods

Drying & Extract Methods	Leaves (µg/g powder extracted in 100ml water)					Bark (µg/g powder extracted in 100ml water)				
	As. Acid	Nico	Ribo	Pyri	Thia	As. Acid	Nico	Ribo	Pyri	Thia
Drying under sun and extracted 1g sample with 100ml water at 30 °C.	4960	50	320	1500	280	2450	15	130	679	132
Drying under sun and extracted 1g sample with 100ml water at 40 °C.	5010	55	342	1660	288	2480	20	133	691	134
Drying under sun and extracted 1g sample with 100ml water at 60 °C.	5300	70	351	1710	300	2740	19	140	679	148
Drying in shade and extracted 1g with water at 30 °C.	5020	55	336	1590	280	2590	15	131	700	141
Drying in shade and extracted 1g with water at 40 °C.	5130	61	351	1660	299	2670	21	141	722	148
Drying in shade and extracted 1g with water at 60 °C.	5410	78	372	1850	318	2290	29	160	80	165

CONCLUSION

Moringa is one of these plants containing plentiful amount of various water soluble vitamins. The current study has explored some of the pharmacognostic and phytochemical aspects of moringa leaves and bark. From results of these studies it is concluded that the leaves microscopy have shown upper epidermis, xylem, phloem and the lower epidermis layers. The different layers are composed of different cells and granules like starch granules. The bark microscopy have also shown three distinct layers like Cork, Cork Cambium and Secondary Cortex with Calcium oxalate crystals, Starch granules and oil globules.

The FTIR spectrophotometry and UV-visible spectrophotometry have shown distinct spectra for powder and extracts samples respectively. It has also been concluded that extracts in different solvents give different UV spectra with varying absorption maxima indicating that quantification by UV method may not produce equal and authentic results in different solvent extracts. Thin layer chromatography (TLC) has separated two vitamins in the samples i.e. vitamin B2 and B6. The high performance liquid chromatography (HPLC) studies have shown that the technique can be used effectively for identification of water soluble vitamins (Ascorbic Acid, Thiamine, Riboflavin, Nicotinamide and Pyridoxine) in moringa leaves and bark extracts. The Rf (TLC) and Rt (HPLC) values of vitamins in moringa leaves and bark have been compared with that of the reference standards and reported in the results section.

The USP-41, HPLC method has been applied for quantification of water soluble vitamins in water extracts of moringa leaves and bark. The method has been properly verified in terms of specificity, linearity, precision and accuracy before using for quantification purpose. The assay results have shown that all the extracts contain ascorbic acid, thiamine, riboflavin, Nicotinamide and pyridoxine in varying concentrations. Generally the vitamins content are higher in amount in samples of Punjab, followed by KPK, Sindh and Balochistan. This may be attributed to the difference in fertility of different provinces land. The results have also shown that leaves samples are more enriched in vitamins content than the bark samples. This observation in consistent with other published studies [29].

The results of the current investigation have shown that drying process generally increase the concentration of vitamins in the plant material [30]. Drying in shade has been shown to be more effective method as drying

under sun destroy some amount of the vitamins. The increase in temperature and shaking during extraction have been seen to increase the extraction (amount) of water soluble vitamins. Further upto 60 ^{o}C temperature no apparent destruction of vitamins has been reported. The amount of vitamins extracted in 5 hrs with 30 ^{o}C heat is approximately similar to that of 24 hrs maceration without any heat, however, at higher temperature the amount of vitamins extracted is more profound.

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