Phyto-Pharmacognostical and Hypocholesterolemic Activity of *Morus alba* L.

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Abstract:

Present studies serve as a basis for determining authenticity as well as a way to distinguish it from other species of *Morus* and develop novel hypocholesterolemic drug. In this work, pharmacognostical, phytochemical and hypocholesterolemic study of *Morus alba* leaves are described. Under pharmacognostical study, macroscopy and microscopy were conducted. Stomatal numbers, stomatal index, palisade ratio, vein-islet numbers and vein termination numbers were studied as leaf constants.

The extraction of dried *Morus alba* leaves powder entails employing a variety of organic solvents, including chloroform, water, petroleum ether and alcohol. Preliminary phytochemical screening was done to find out plant metabolites such carbohydrates, fats, proteins, alkaloids, saponins, glycosides, coumarins, tannins, flavonoids, triterpenoids, amino acids, phytosterols, resins, oils and phenols. HPLC and TLC is a techniques used for identification and quantification of physiologically active substances. *Morus alba* has rutin content 1.20 mg/g, quercetin content 1.35 mg/g, chlorogenic acid 2.01 mg/g and kaempferol 1.10 mg/g . These phytoconstituents shows antidiabetic, antioxidant and hypocholesterolemic activity. The hypocholesterolemic activity was conducted in male SD rats. Rats were administered Triton X-100 intraperitoneally to induce hypercholesterolemia. The hydroalcoholic extract dose of 300 mg/kg body weight and standard compound atorvastatin dose of 10 mg/kg body weight were administered orally. Test medication reveals a decrease in total cholesterol, triglycerides and low-density lipoprotein while increasing high-density lipoprotein.

Keywords:- *Microscopic, Physico-chemical, TLC, HPLC, Hypocholesterolemic*

INTRODUCTION

Both industrialized and developing nations use herbal items frequently as household remedies. Herbal drugs are one of the sources which are used for the treatment and management of various diseases. The creation of herbal medications and phytopharmaceutical chemicals has been based on the utilization of plant extracts and isolated pure components. A healthcare product's degree of purity, potency, efficacy and safety must be determined scientifically in order for it to be widely recognized [1,2].

The World Health Organization has established normative criteria, including physicochemical and phytochemical examination of crude pharmaceuticals, to evaluate the quality, purity and effectiveness of herbal plants. Setting these pharmacognostical standards involves a number of processes in order to create a monograph for a basic medication. To demonstrate why medicinal plants are acceptable in the conventional medical system, it is crucial to evaluate their quality. The use of standards, which are numerical attributes by which the quality of herbs may be evaluated, promotes uniformity of quality [3,4,5].

White mulberry is scientifically known as *Morus alba* and belonging to family moraceae. It is also known as Shahtoot in Hindi, Tuta in Sanskrit, Tuti in Marathi and Toot in Persian. China is the native home and grown all throughout India's plains and up to an elevation of 3300-3500 meters in the Himalayan foothills. It is a medium-sized monoecious shrub that is occasionally dioecious [6,7]. Mulberry is primarily grown for its leaves, which are fed to silk worms. The white mulberry is well-known for its therapeutic benefits. The northern hemisphere's tropical and subtropical zones are home to mulberries. India is home to four or five different mulberry species. Mulberry leaves can be elliptical, widely ovate, crenate-serrate, or deeply lobed, and they can also be serrated or not. In traditional Chinese medicine, numerous plant components, including the roots, stems, leaves, flowers, barks, fruits, seeds and branches, have been utilized to cure a variety of ailments [8,9,10].

It serves as an emetic, diuretic, hypoglycemic, hypolipidemic, anti-inflammatory, antihelmentic, antioxidant, expectorant and laxative. Infections caused by viruses, malaria, rheumatoid arthritis and fever are also treated with it (1999; Zhishen; 2003; Kim). *Morus alba* leaves are used as an anti-hyperglycemic meal in Japan and Korea (Matsuoka 1994). Mulberry leaves

are rich in flavonoids and shows antioxidant activity [11,12,13].

From mulberry leaves, a glycoside with a few prenylflavanes and benzyl-glucopyranoside were extracted, and it was discovered that these compounds have hypocholesteromic activity and might prevent atherosclerosis. Rats that were given alcoholic mulberry leaf extracts had significantly less fat buildup. *Morus alba* is generally cultivated for its leaves to feed silk worms in China and for fruits in Europe [14,15,16].

Taxonomical Classification

Kingdom	Plantae
Phylum	Spermetophyta
Division	Angiospermae
Class	Dicotyledons
Order	Rosales
Family	Moraceae
Genus	*Morus*
Species	*Alba*

MATERIAL AND METHODS

Collection and Authentication of Plant Material:

Morus alba (white mulberry) have been collected from IET campus Lucknow, Utter Pradesh in March. The authentification has been done with the help of CSIR-NBRI, Lucknow, Utter Pradesh. The sample confirmed as *Morus alba* and accession No. is LWG -100982.

Pharmacognostical Analysis:

Macroscopical Examination

The easiest method for using sense organs to identify specific herbs is organoleptic evaluation. Shape, size, colour, scent, taste, base, apex, margins of leaves exterior and evaluation of venation are organoleptic [17,18]. *Morus alba* is a medium-sized deciduous tree, it can reach heights of 15 m and has a girth of 2–3 m. The plants look is rounded or bushy because to the 30-45 cm tall stem and more dispersed branches. The bark is dark grayish brown, tough with vertical fissures [19,20].

Flowers are greenish and sometimes the sexes are found on different branches of same tree or different trees. Male spikes are hairy, 2.5mm long, roughly cylindrical or ovoid, and range in length from 1.3 to 3.8 cm. Female spikes are ovoid, pedunculate, with four sepals that are either glabrous or briefly ciliate and a catkin inflorescence [21,22].

Microscopical Examination of Leaf:

Qualitative Microscopy

Studies at the macroscopically level are crucial for separating closely related plant species so microscopical examinations are essential for separating to each other. The *Morus alba* leaf was cut into sections to demonstrate the structure and organization

of the tissue system. With the aid of a wax-embedded blade, thin portions are sliced. Every leaf section was placed in the water-filled watch glass. For the dewaxed entire portions, xylene was utilized. All sections were stained using safranin. A drop of glycerin was applied to the slide after staining. Choose the extremely fine part and then place on the glass slide. A cover slip that was placed on the glass slide lacking any air bubbles being trapped inside [23].

Quantitative Microscopy

Determination of Leaf Constants

Several leaf measures can be used to differentiate between some closely related plant species. Peeling the lower and upper epidermal surfaces of the leaf, washing them with chloral hydrate solution and examining the leaf surfaces under a microscope to check for stomatal numbers, stomatal index, palisade ratio, vein-islet numbers and vein termination numbers were studied as leaf constants [24].

Stomatal Number

Chloral hydrate solution was taken to clean the leaf surface. With the use of forceps, the lower and upper epidermis were separated individually and put on a glass slide being mounted in glycerin. Draw a 1mm square using a stage micrometer and count the number of stomata in that square using a camera lucida. Calculate the average number of stomata per square millimeter after recording the result ten times [25].

Stomatal Index

Surface of the leaf was cleaned by using potassium hydroxide solution and with the help of forceps, the upper and lower epidermis were separated individually. The upper and lower epidermis put on a glass slide being mounted in glycerin. Use of a micrometer and camera lucida, a square with 1 mm sq was created and the quantity of epidermal cells and stomata were counted in the 1mm sq region. The average was obtained after ten determinations were made. The following equation was used to determine the stomatal index [26].

Stomatal index **(S.I.) = (S/ E+S) × 100**

Where,

S = number of stomata per unit area

E = number of epidermal cells in the same unit area

Vein-islet Number

A piece of the leaf was cleared by boiling in potassium hydroxide solution for around 30 minutes. The camera lucida and drawing paper were set up and 1 sq mm drowns using a stage micrometre. Count the number

of vein- islet present within the square and divide the total vein- islet number in 4 adjoining squares by 4 to obtain the value of 1 mm sq. 10 sets of 2 mm by 2 mm areas were used to calculate the average [27].

Veinlet Termination Number

The same preparation used to count the number of veinlet termination. To obtain the value in 1 sq mm, the total veinlet termination number in 4 adjacent squares was divided by 4. 10 sets of 2 mm by 2 mm areas were used to calculate the average[27].

Palisade Ratio

With the use of forceps, the 2 mm square middle portion of the *Morus alba* leaf was cleaned by boiling in a potassium hydroxide solution before being placed in glycerin. In order to trace the palisade cells and epidermal cells a camera lucida was set up. First the outlines of many groups of each of the four epidermal cells were traced. Then focused and traced the palisade cells beneath each group. Every groups of palisade cells that covered by more than half epidermal cells were counted and the total was divided by four to determine that group palisade ratio. The determination of 25 groups from various leaf samples was used to compute the average [24].

Powder Microscopy

Powdered raw *Morus alba* leaf material is processed and cleaned with potassium hydroxide solution. A clear slide was taken and inserts a drop of glycerin water in the middle of the glass slide. Powdered drug is placed on the glass slide and a cover with the cover slip no air bubbles present and then observed under microscope [24].

Phytochemical Analysis:

Phytochemical Screening of different extracts

50 mg of powdered leaves were extracted in the soxhlet apparatus. To obtain crude extracts, a variety of organic solvents are used, ranging from polar to non-polar grade (water, ethanol, chloroform and petroleum ether). All four extracts were dried using a rotary evaporator and the dried extracts were stored in a desiccator for later use. The presence and absence of different phytoconstituents such alkaloids, glycosides, carbohydrates, steroids, proteins, tannins, resins, etc. were examined in all extracts [28,29].

Thin Layer Chromatography

Morus alba leaf extracts were examined for the presence of numerous chemical components using thin layer chromatography (TLC). For TLC, silica gel was utilized as the adsorbent. A coated TLC

plate was heated in the oven for 30 minutes at 110^0C to activate it. The standard compounds rutin, quercetin, chlorogenic acid, kaempferol and gallic acid were dissolved in ethanol and applied on centre of the glass plate using capillaries [30]. The TLC plate was kept in the development tank with a solvent system. The plates were left to air dry at ambient temperature for around 20 minutes, after which they were sprayed with 1% sulfuric acid and the spots seen under UV light at 366 nm and determined Rf values [31,32].

HPLC Analysis

HPLC is an analytical method used for identification and quantification of the various phytoconstituents of the extracts. It's chromatogram show the occurrence of various compounds in the plant sample [33].

Sample Preparation

Under ultrasonic exposure (30.0×25.0×12.5 cm, 34±3 kHz, PZT sandwich-type six transducer, 250 W, Oscar Micro clean-109, Mumbai, India), 100 mg of the dried powder was exposed to solvent (3x10ml, water: methanol -20:80 v/v) at 40^0C. Undissolved substances were separated by centrifugation [34,35]. The combined organic solvents were concentrated under vacuum before being redissolved in a 10 mL eppendorf with methanol. Before HPLC-PDA (High Performance Liquid Chromatography-Photodiode Array) analysis, samples were filtered using a 0.45 m nylon filter membrane. Methanol was used for the preparation of standard rutin, quercetin, chlorogenic acid and kaempferol solution1mg/ml [36,37].

HPLC Finger Print

Shimadzu HPLC System was used which has an injector, column, binary pumps and a detector (PDA) to create a chromatographic fingerprint. Using a Phenomenax C18 Luna (250'4.6mm, 5m) column for partition of phytoconstituents was accomplished using an optimized solvent mixture of methanol and acidified water [38,39]. With dual solutions A (acetic acid in water, 1% v/v) and B (methanol 100%), the HPLC linear gradient elution programme: The following parameters were chosen: 0 min, A 95% and B 5% 15 min, A 70% and B 30% 30 min, A 60% and B 40% 60 min , A 50% and B 50% 70 min, A 45% B and 55% 90 min, A 0% B and 100% to maintain the 1.0 ml/minutes flow rate during process [40,41]. To create an HPLC fingerprint, a 10µl injection volume, a 30^0C

thermostated column, photo-diode array (PDA) detection in the 200–800 nm region and 254 nm data were used. Rutin, quercetin, chlorogenic acid and kaempferol was identified by retention time and matching the UV-spectra of sample [42].

Hypocholesterolemic Activity:

***Animal Selection*:**

7-8 week old male SD rats were used to test the hypocholesterolemic effect. The rats were kept in polypropylene cages with 23-20°C temperature, 50–60% humidity level and 300 lux light intensity. Test animals (SD rats) were given access to food and water at will. Test animals were given a single intra-peritoneal injection of triton-X-100 (100 mg/kg in normal saline solution) to make them hypercholesterolemic after 12 hours of total fasting. The test animals divided into 4 groups with 6 test animals in each group. The conventional medication Atorvastatin (10 mg/kg) and leaf extract at a dose of 300 mg/kg body weight dissolved in saline solution were administered orally to SD rats [24,43].

Group1-Control group (normal saline solution)

Group2-Freshly prepared solution of Triton-X-100, injected intra-peritoneally

Group3-Standard drug Atorvastatin administered orally (10 mg/kg).

Group4- Orally administered with dose of 300 mg/kg hydroalcoholic extact (60% water and 40% ethanol) of *Morus alba.*

***Blood Sample Collection*:**

All SD rats that had been fasted overnight had a tiny amount of blood (2-4 ml) taken from them at the end of the week. Blood was drawn from each group's retro-orbital venous plexus using a glass capillary tube and ether as anesthesia. After collecting the blood sample, it was centrifuged to separate the serum, which allowed the blood to clot as usual at room temperature. By analyzing this separated serum using accepted techniques, various parameters including triglycerides (TG), total cholesterol (TC), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) were estimated [44,45].

***Statistical Analysis*:**

Data sets in triplicate were gathered and one way analysis of variance (ANOVA) was carried out to analyze the data and produce results. The result has been examined in terms of mean and SD.

RESULT AND DISCUSSION

Macroscopical Examination:

Its composite fruit is ovoid to ellipsoid-shaped and is made up of many drupes. Fruits are 2-3 cm long. When ripe, they are white to pinkish white in colour (Fig. 1A).

Usually leaves are 5 to7 cm long, alternating, ovate or broadly ovate, simple, acute, crenate-serrate, cordate base and frequently lobed, leaves are quite diverse in shape and size. Young plant leaves have a slight pubescence along the nerves below, lateral nerves that branch towards the border and petiole is typically between 1.8 to 2.5 cm long (Fig.1B).

The macroscopical descriptions including morphological and organoleptic characters of the leaf of *Morus alba* are shown in Table 1.

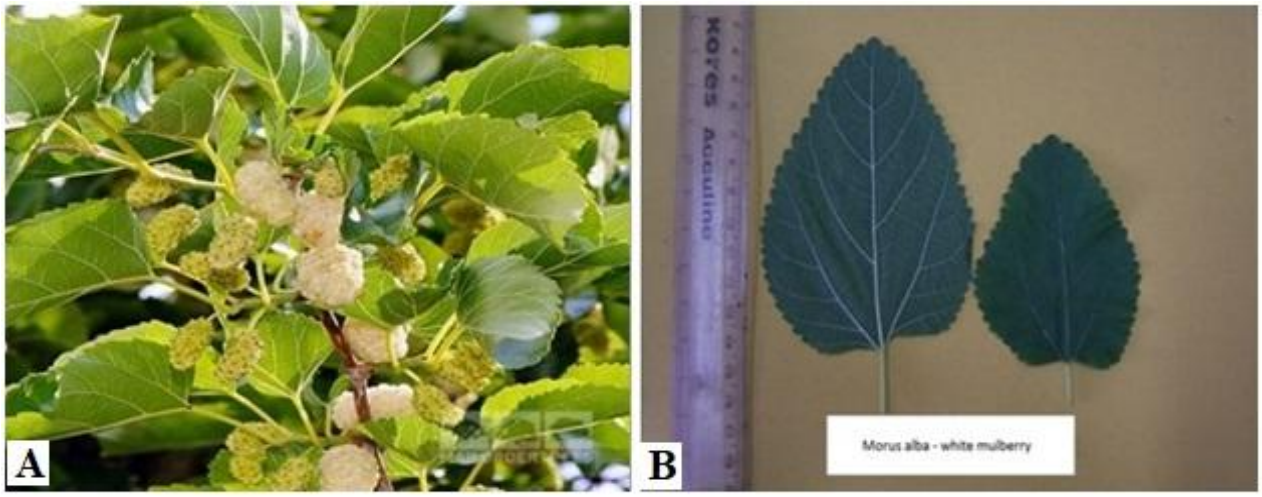

Fig. 1: Macroscopical characters of *Morus alba* **(A)** Appearance of fruits **(B)** Appearance of leaves

Table 1: Macroscopical Discriptios of the Leaf of *Morus alba*

Macroscopical parameters	Leaf discription
Organoleptic characters	
Taste	Characteristic
Odour	Characteristic
Colour of upper surface	Deep green
Colour of lower surface	Light green
Texture	Slightly rough surface

Marphological characters	
Type of leaf	simple, alternate
Length of leaves	5 -7 cm
Width of leaves	2 - 4 cm
Shape of leaves	Ovate or broadly ovate
Margin	serrate or crenate-serrate
Base of leaf	Cordate or truncate
Apex	Acute or shortly acuminate

Microscopical Examination of Leaf

The lower and upper epidermis of *Morus alba* leaf were visible in the transverse section. The upper epidermis is composed of a single layer of subrectangular cells oriented tangentially, each with a thick cuticle and unicellular covering trichomes. Paracytic types of stomata are present. The upper epidermis of a leaf lacks stomata because it is wax-coated. The lamina lowers 5–6 layers of spongy parenchyma and the lamina uppers 1-2 layers of palisade parenchyma. The lower epidermis is made up of a single layer of subrectangular cells with a smooth cuticle that are tangentially oriented (Fig. 2A).

The *Morus alba* leaf petiole section cutting has an oval form and a grove. There are numerous unicellular, thick-walled trichomes present on the surface. Cuticle covers the epidermis. The cortical region is made up of four to five layers of collenchymatous cells. Vascular bundles are organized in a ring in various quantities. Tracheids, vessels, fibres, and parenchyma make up xylem. The thick wall of parenchymatous cells is lignified (Fig. 2B). The phloem covering the xylem externally. Sieve tubes, phloem parenchyma, and companion cells make up the phloem.

The pith is made up of parenchymatous cells with thin walls and 9-10 vascular bundles. Cortex and pith both contain calcium oxalates crystals in rosette shapes (Fig. 2C). Various characteristic feature of petiole presented in table 2.

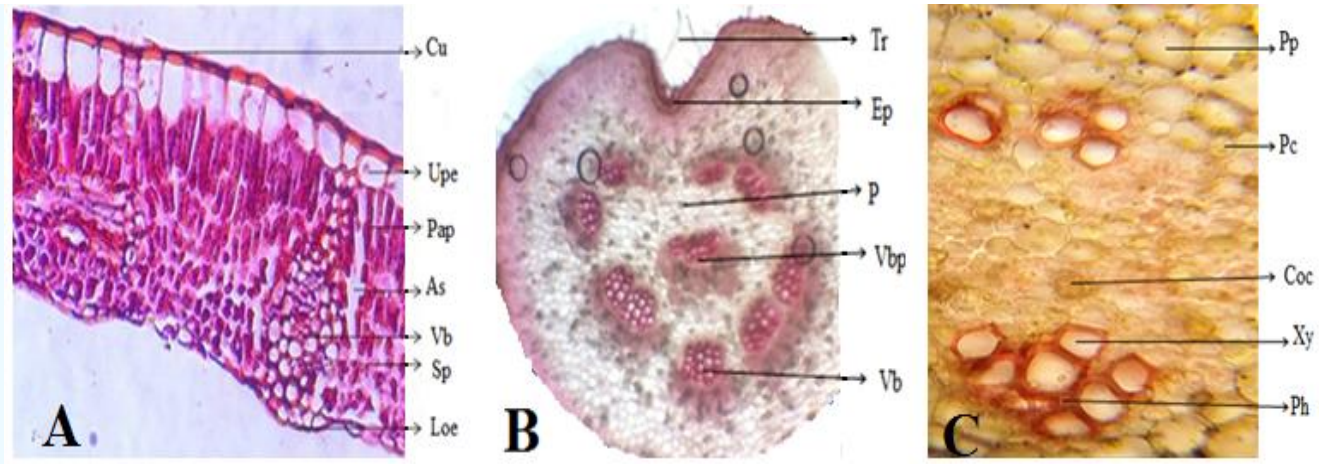

Fig. 2: (A) Transverse Section of *Morus alba* midrib showing: Cuticle(cu), Upper epidermis (Uep), Palisade parenchyma (Pap), Air space (As), Vascular bundles (Vb), Spongy parenchyma (Sp) and Lower epidermis (Lep). **(B)** Transverse Section of *Morus alba* petiole showing: Trichomes (Tr), Epidermis (Ep), Pith (P), Vascular bundles of pith (Vbp), and Vascular bundles (Vb). **(C)** Pith zone showing: Pith parenchyma (Pp), Pith collenchyma (Pc), Crystals of calcium oxalate (Coc), Xylem (Xy) and Phloem (Ph).

Table 2: Different Characters of Petiole of *Morus alba*

Characters	Results
Covering Trichomes	Present
Glandular Trichomes	Absent
Starch Grains	Absent
No. of vascular bundles in pith region	Only one
Crystals of calcium oxalate	Rosette type, abundant in pith and cortex

Quantitative microscopy

The quantitative microscopy of *Morus alba* leaf such as stomatal number, stomatal index, palisade ratio, number of vein-islets and number of vein terminations are diagnostically important, presented in Table 3.

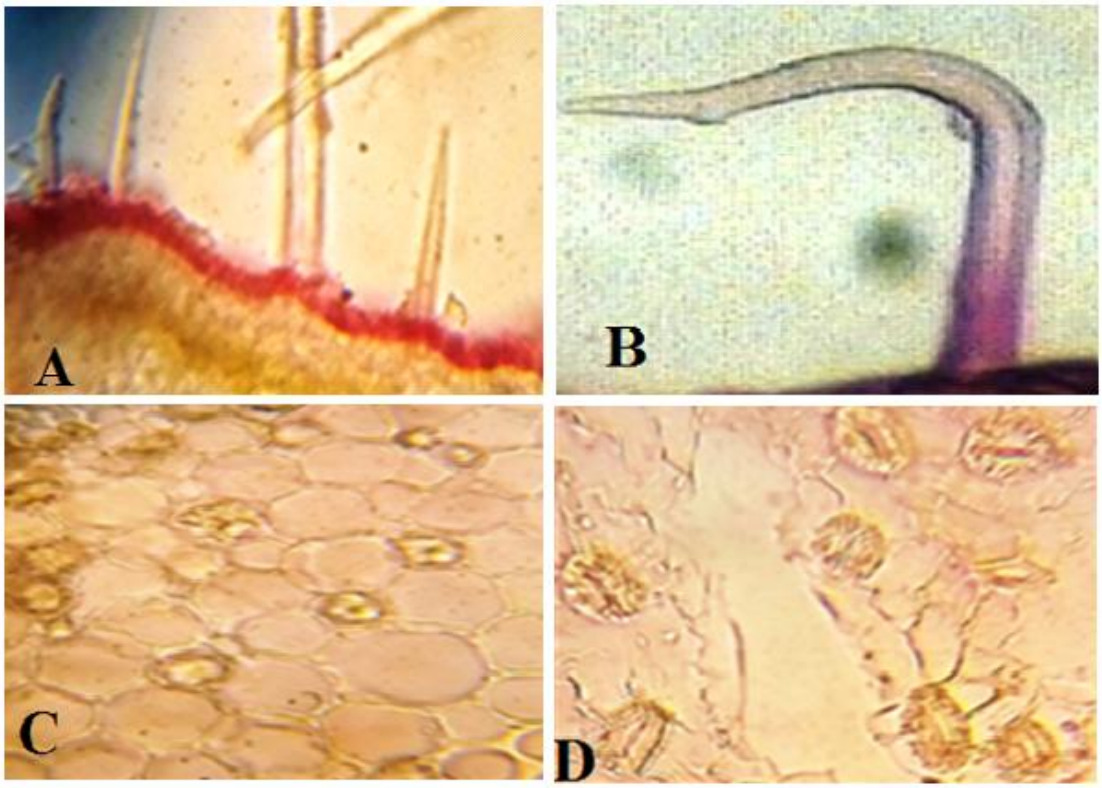

Fig. 3: powder microscopy of leaf **(A)** covering trichomes **(B)** curved shaped trichome **(C)** Calcium oxalate crystals **(D)** paracytic type stomata.

Table 3: Quantitative microscopy of *Morus alba* leaf

Parameters	Values
Stomatal number	15
Stomatal index	11.28
Palisade ratio	14.65
Vein-islet number	5
Vein termination number	2

Powder microscopy of *Morus Alba*

The powder microscopical constituents of *Morus Alba* including types of trochomes, calcium oxalate crystals and types of stomata are presented in Fig. 3 A-D.

Preliminary Phytochemical Analysis

The presence and absence of secondary metabolites in various extracts (Petroleum ether, chloroform, ethanol, and water) of *Morus alba* are presented in Table 4.

Table 4: Phytochemical Analysis in Various Extracts

Test for alkaloids	**Petroleum ether**	**Chloroform**	**Ethanol**	**Water**
Wagner's test	+	-	-	-
Mayer' test	+	+	-	-
Dragendroff' test	-	+	+	+
Hager's test	+	+	-	-
Test for sugars				
Barford's test	+	-	+	+
Tollen's test	-	+	+	+
Molish's test	+	+	+	+
Benedict's test	_	+	-	-
Fehlig's test	+	+	-	-
Test for glycosides				
Legal's test	-	-	+	+
Keller kiliani's test	+	-	+	+
Baljet's test	+	+	+	-
Borntrager's test	+	+	+	+
Test for sterols				
Liebermann's test	-	-	-	+
Liebermann-Burchard's test	-	+	+	+
Salkowaski's test	+	-	+	+
Test for proteins and amino acids				
Millon's test	-	-	-	+
Ninhydrin test	+	+	+	-
Biuret test	-	+	+	-
Xanthoproteic test	+	+	-	-
Test for saponin				
Foam test	-	-	+	+
Hemolytic test	+	+	+	-
Test for flavonoids				
Lead acetate test	+	+	-	-
Shinoda test	-	+	+	+
Ferric chloride test	+	+	+	+
Test for tannins				
Match stick test	+	+	-	-
Gelatin test	+	+	-	+
Lead acetate test	+	+	-	-
Ferric chloride test	+	+	+	+
Bromine water test	-	-	+	+
Potassium dichromate test	+	-	-	-
Test for coumarin	-	+	+	+
Test for phenols				
Lead acetate test	+	+	-	-
Ferric chloride test	+	+	+	+
Test for gums and mucilage	-	-	-	-
Test for oil and fats				
Spot test	+	+	+	+
Saponification test	+	+	+	-
Bromine water test	-	-	+	+

Thin Layer Chromatographic analysis

The Rf values of standard compounds like rutin, quercetin, chlorogenic acid, kaempferol and gallic acid were 0.58, 0.95, 0.74, 0.87 and 0.35 respectively. The number of spots is higher in petroleum ether. Rutin, quercetin, and gallic acid can be seen in petroleum ether extract. Chlorogenic acid, kaempferol and gallic acid are present in chloroform extract. The presence of quercetine is revealed by ethanol extract. Rutin and gallic acid are present in a water extract. Values of R_f given in Table 5.

HPLC Analysis

Retention time of quercetin 2.913, rutin 39.785, chlorogenic acid 18.485 and kaempferol was observed at 86.169 min.

The HPLC and 3D chromatograms are shown in Fig. 4 A and B. The UV-spectra of all phytoconstituents was analyzed at 254 nanometer (Fig. 4 A-D).

Morus alba was found to have rutin content 1.20 mg/g , quercetin content 1.35 mg/g, chlorogenic acid 2.01 mg/g and kaempferol 1.10 mg/g are represented in Table 7.

Table 5: TLC Analysis of various extracts

Extracts	Mobile phase	No. of spots in UV light	R_f values
Ethanol	methanol : Ethyl acetate (20:80)	3	0.62, 0.95, 0.71
Chloroform	Ethyl acetate : Toluene (7:93)	3	0.35, 0.74,0.87
Petroleum ether	Ethyl acetate: toluene: diethyl amine (20:70:10)	5	0.35, 0.42 0.58, 0.62, 0.95
Water	Formic acid: ethyl acetate: Water : glacial acetic acid (11:100: 26:11)	2	0.35, 0.58

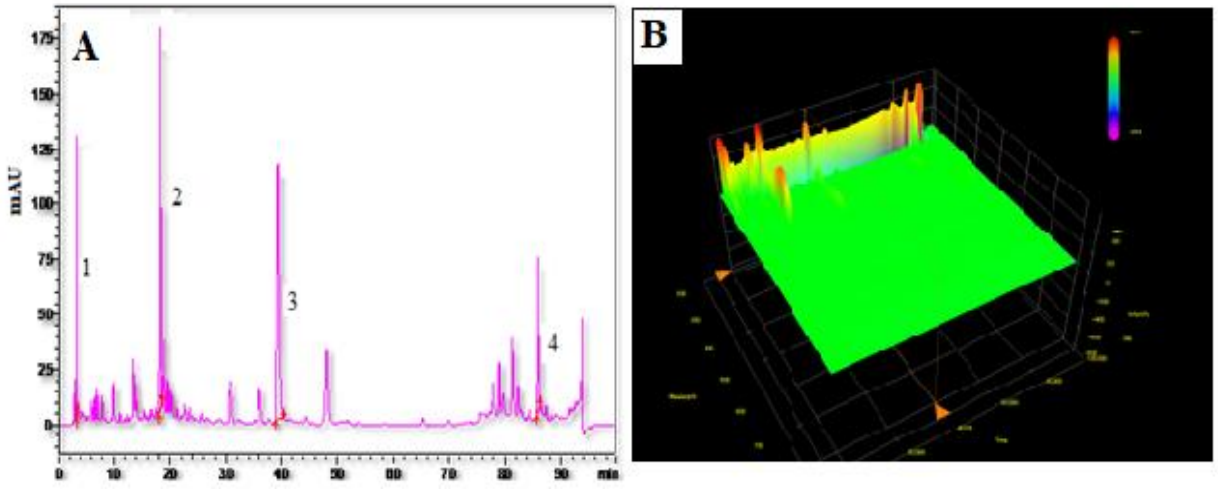

Fig. 4: (A) HPLC Chromatogram (B) HPLC 3D Chromatogram

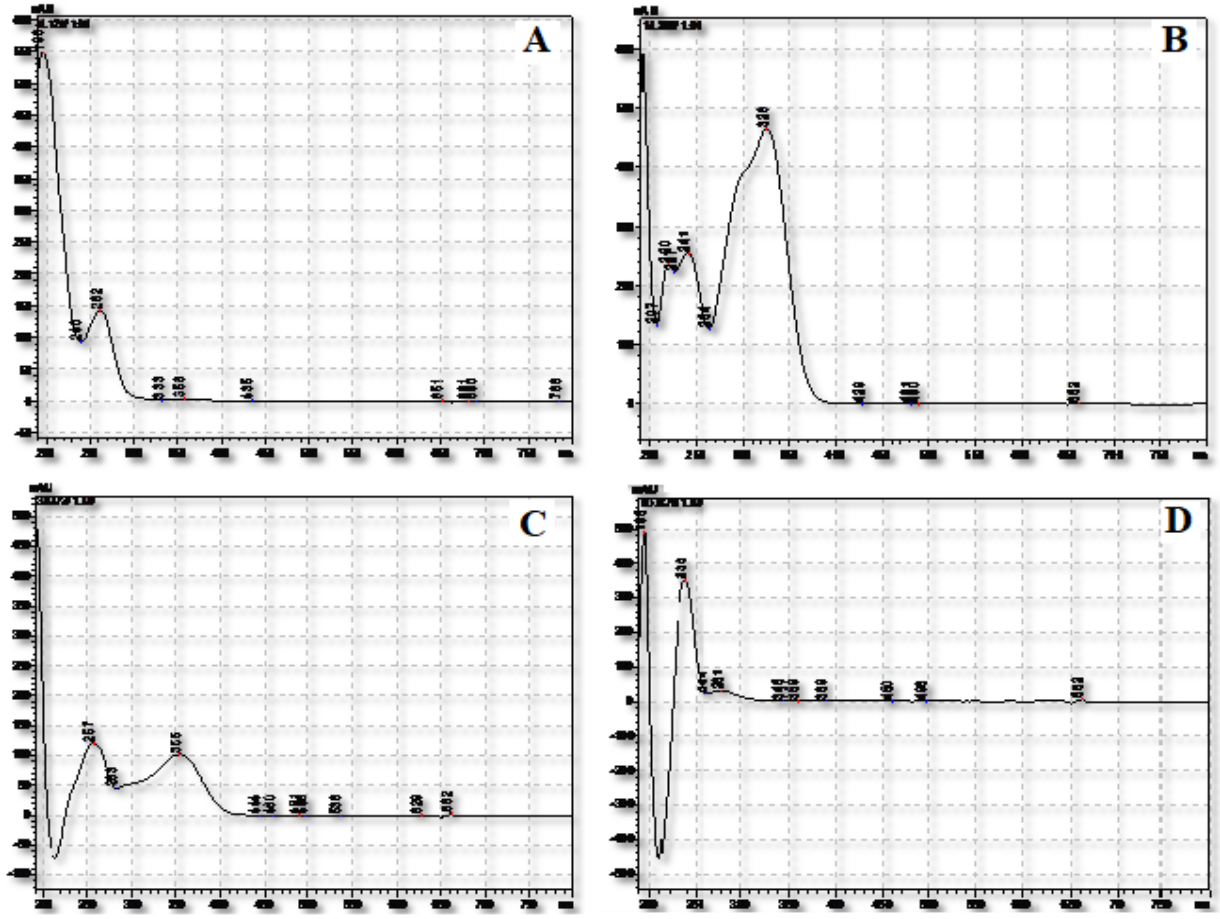

Fig. 5: (A) UV Spectra of Peak-1 (B) UV Spectra of Peak-2 (C) UV Spectra of Peak-3 (D) UV Spectra of Peak-4

Table 6: Quantity of Phytoconstituents

Sample Concentration (100mg/ml)	
Phytoconstituents	Amount in (mg/g)
Rutin	1.20
Quercetin	1.35
Chlorogenic acid	2.01
Kaempferol	1.10

Hypocholesterolemic Activity:

The results demonstrate that high-density lipoprotein levels fell in the Triton X-100 (100 mg/kg) treated control group while serum cholesterol levels increased. Standard dose of atorvastatin (10 mg/kg) significantly decreased blood cholesterol levels. Plant extracts (300 mg/kg) significantly decreased the level of serum cholesterol. Total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) were all dramatically decreased, while HDL was markedly elevated (Table 7).

Table 7: Effect of hydroalcoholic extract on serum lipid level in SD rats

Groups	**Normal Control**	**Diseased Control**	**Standard Control**	***Morus alba***
HDL (mg/dl)	18.12±4.49	17.18±2.45	24.85±3.47	21.95±3.15
LDL (mg/dl)	85.62±3.15	89.62±3.15	57.25±3.38	63.25±2.82
TC (mg/dl)	129.85±2.25	137.85±2.25	97.27±1.93	101.7±2.31
TG (mg/dl)	108.65±3.63	119.65±3.63	75.32±2.52	78.17±3.54

Conclusions:

The *Morus alba* leaf is the subject of this essay, which focuses on its morphological characteristics, organoleptic characters, chemical composition, physicochemical parameters and microscopical characteristics. Macroscopical characters are not sufficient to identify closely related plant species so all parameters are used to identification of closely related plant species. To identify the drugs for pharmacologically active ingredient, phytochemical research is also helpful. It aids in judging the purity and quality of plant-based medicines. The hydroalcoholic extract of *Morus alba* reveals a decrease in

total cholesterol, triglycerides and low-density lipoprotein while increasing high-density lipoprotein. These outcomes may serve as a basis for determining authenticity as well as a way to distinguish it from other have no adverse effects, the majority of people rely on herbal products.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article

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