

Contents lists available at UGC-CARE

International Journal of Pharmaceutical Sciences and Drug Research

[ISSN: 0975-248X; CODEN (USA): IJPSPP]

journal home page: http://ijpsdronline.com/index.php/journal



Research Article

Standardization and Hypolipidemic Effect of Fraxinus micrantha Leaf

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ARTICLE INFO

Article history:

Received: 28 December, 2024 Revised: 08 March, 2025 Accepted: 12 March, 2025 Published: 30 March, 2025

Keywords:

Hyperlipidemia, *Fraxinus* micrantha, hypolipidemic effects, High fat diet, Lipid profile.

DOI:

10.25004/IJPSDR.2025.170204

ABSTRACT

Hyperlipidemia, characterized by elevated levels of various lipid fractions in plasma, is a significant risk factor for cardiac conditions such as coronary artery syndrome, stroke, and cardiac arrest. Traditional treatments often involve synthetic hypolipidemic drugs (such as statins, fibrates, and niacin) which can have adverse effects (like muscle pain, liver damage, and digestive problems). Consequently, there is a growing interest in natural alternatives for managing hyperlipidemia. Misidentifying or adulterating plant material can compromise the effectiveness of medicinal plants. Evaluating the pharmacognostic parameters is essential in ensuring the authenticity and quality of these plants. Fraxinus micrantha, a plant used traditionally in the Himalayas for its therapeutic properties, was investigated for its potential hypolipidemic effects. The study involved collecting and analyzing the leaves of F. micrantha for their phytochemical composition and hypolipidemic effects in a Wistar rat model produced by a diet rich in fat. A total of six male Albino Wistar rats of weight 190 ± 15 g each were used to construct test, atorvastatin standard, and high-fat diet (HFD) control groups. Organoleptic, microscopic, and physicochemical evaluations confirmed the plant's quality and composition. The extracts at 200 and 400 mg per kilogram of body weight orally were assessed for their impact on lipid profiles, showing that both F. micrantha aqueous (FMAQ) and methanol extracts (FME) significantly reduced serum cholesterol, triglycerides, and LDL levels while increasing HDL, with FME showing superior efficacy compared to FMAQ. The study highlights the potential of F. micrantha as a natural hypolipidemic agent, offering a promising alternative to synthetic drugs.

INTRODUCTION

The disorder of lipid metabolism known as hyperlipidemia, which causes heart disease, is caused by an increase in the amount of lipids (fats) or lipoproteins in the blood. It is defined as increased various lipid and lipoprotein fractions, such as triglycerides (TG), total cholesterol (TC), LDL, VLDL, and even high-density lipids (HDL) in some cases which are linked to a number of problems, including coronary artery disease, stroke, myocardial infarction, atherosclerosis, pancreatitis, and heart attacks. [1] There are two types of hyperlipidemia: primary and secondary. Drugs that lower cholesterol can treat the former, but the latter type caused by hypothyroidism, diabetes, or renal lipid nephrosis can be treated by treatment focusing on addressing the primary condition itself. [2] Worldwide,

dyslipidemia is mostly caused by genetic diseases and a lifestyle diet high in calories, fat, and cholesterol. One of the primary causes of hyperlipidemia is dietary changes, with poor diet being the primary risk factor. This includes getting over 10% of calories from fat, and over 40% of calories from fat along with over 300 mg of cholesterol every day.

There are many synthetic medications available for hyperlipidemia; however, none of them are particularly effective for treating lipoprotein abnormalities, and they are all associated with an array of side effects. ^[5] There are numerous allopathic hypolipidemic medications on the market, such as statins, but they have several negative side effects, including hyperuricemia, diarrhea, myositis,

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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hepatotoxicity, and others.^[6] As a result, alternative materials are currently being sought after from natural sources to reduce their toxicity, lower their cost, and offer better long-term safety and effectiveness. Plant-based natural products have long been utilized in medicine to cure a variety of illnesses.^[7]

One member of the Oleaceae family of olives, which is primarily found in India and Nepal, is Fraxinus micrantha.[8] In India, it is typically found in the Himachal Pradesh and Uttar Pradesh provinces. Since the beginning of time, F. *micrantha* has been investigated for its potential medical and financial benefits worldwide. [9] The native people of Dharchula, Himalayas, have long used the plant's inner bark as an infusion to treat liver issues such as jaundice and enlargement.[10] Owing to the inclusion of coumarin glycoside, a strong diuretic agent, and a number of glycosides, including fraxin. The bark and leaves are also used to treat itchy scalps, rheumatoid arthritis, bowel movements, and cystitis. Fraxinus plant is renowned for its diverse pharmacological characteristics including neuroprotective, anti-inflammatory, antioxidant, and anticancer. [11] The leaf extract's triterpenoid content, which includes esculetin and ursolic acid supported its use in the treatment of amoebic dysentery. [12] A recent study showed the prominent effect of *F. micrantha* leaf extract on obesity.[13]

This investigation attempts to assess the hypolipidemic impact of *F. micrantha* extract in Wistar rats that have been made hyperlipidemic by a high-fat diet while also assessing the plant's phytochemical composition, physico-chemical properties, and safety profile through organoleptic, microscopic, and contamination analysis. The standardization of herbal drugs is a complex undertaking, as multiple factors can impact both the bio-efficacy and the reproducibility of therapeutic effects. Therefore, it is imperative to ensure that the herbal product obtained is of the highest quality. [14]

MATERIAL AND METHODS

Collecting, Identifying and Authenticating Plant Material

In May 2024, fresh *F. micrantha* Lingelsh leaves were collected from Nainital, which is in the Kumaun area of the Himalayas in India. Plant samples were submitted for identification and authentication to the Vital Herbs, Uttam Nagar, Delhi India. After being allowed to dry in the shade at ambient temperature, the leaves were ground up.

Testing Agents

Each component utilized in this investigation was supplied by Hi-Media Lab Pvt. Ltd. (India), Sigma-Aldrich Chem Co. (USA), SD Fine-Chem. Ltd. (India), and SRL Pvt. Ltd. Only analytical-grade substances were utilized in this investigation.

Organoleptic Evaluations

By utilizing our natural senses- sight, taste, smell, and touch detailed information about a plant's attributes such as size, taste, texture, color, shape etc., were identified. [15]

Microscopic Investigation

The fresh leaves were collected and fixed immediately for 24 hours in the FAA solution that contained acetic acid, formalin, and 70% alcohol in the proportion 5:5:90 to prevent shrinkage of cells. After that, the leaf is cut into smaller pieces and transformed into wax blocks by embedding them in a paraffin. The transverse sections of the leaf were made by using a sharp blade. [16] The detailed study of cellular and tissue structures of sections was observed using the compound microscope. [17]

Powder Study

To facilitate microscopic inspection, the crude medication was ground into a powder and combined with 75% chloral hydrate. After mounting a pinch of moistened powder on the glass slide and covering it with a glass cover slip, the prepared slide was inspected under a light microscope to find the tissue and cellular structures, then pictures with a digital camera. [18]

Physico-chemical Analysis

The normal techniques were followed to determine the physicochemical characteristics, such as the ash value (total ash, water-soluble ash, and acid-insoluble ash), and loss on drying. Using a slightly modified version of the specified process, the extractive values were determined using the cold maceration technique. [19] After precisely weighing 5 g of the coarse powder of *F. micrantha*, it was placed in three conical flasks with glass stoppers, each containing 100 mL of the solvents (alcohol, methanol, and water). The mixture was macerated in a closed vessel for approximately 6 hours with the aid of a mechanical shaker. and it was left to rest for 18 hours at room temperature (24 ± 2°C). The extracts were dried in an oven set at 105°C after being filtered using Whatman No. 1 filter paper that had been reduced using a rotary evaporator. [20] The extracted value in percentage w/w for each solvent was determined using the weight of the residue that was collected.

Determination of Foreign Matters

The extracts were dried in an oven set at 105°C after being filtered using Whatman No. 1 filter paper that had been reduced using a rotary evaporator. The extracted value in percentage w/w for each solvent was determined using the weight of the residue that was collected [21].

Estimation of Contamination Parameters

Pesticide content

Pesticide analysis of leaves was performed following WHO standards. About 50 g of crushed leaves were mixed with

350 mL of acetonitrile and 135 mL of water. The mixture was filtered, and 250 mL of the filtrate was blended with 100 mL of light petroleum, 10 mL of sodium chloride solution (40%), and 600 mL of water for 5 minutes. The organic layer was separated, washed with water, and concentrated to 5 to 10 mL. The concentrated extract was passed through a Florisil column heated to 650°C, yielding three eluates. Chloride-based and phosphate-based pesticides were detected using specific absorbance measurements at 460 and 820 nm, respectively. [22]

Heavy metal analysis

Heavy metals (mercury, lead, zinc, cadmium) were analyzed by digesting 2 g of powdered sample with 10 mL concentrated nitric acid, followed by heating at 95°C. After repeated digestions, the solution was cooled, treated with 2 mL deionized water and 3 mL $\rm H_2O_2$, and then further treated with hydrochloric acid. The final volume was adjusted to 50 mL, and heavy metal concentrations were measured using atomic absorption spectroscopy (Shimadzu AA6300), expressed in ppm per gram of the sample. [23]

Evaluation of aflatoxin

HPLC was employed to evaluate plant extracts for B1, B2 and G1, G2 aflatoxins in accordance with the AOAC Official Method (2000 and 2006). The evaluation was carried out on Typical HPLC system based on the Perkin Elmer Ser. 200 that had a UV detector with an autosampler. A 25 cm x 4.6 mm i.d. C-18 column with a mobile phase made up of methanol, acetonitrile, and water in a 57:17:26 ratio was used to accomplish separation. The rate of fluid flow was preset at 0.5 mL/min, and the detection process was carried out at 365 nm. $^{[24]}$

Analysis of microbial contamination

The present study delved into the examination of microbial contamination in various plant extracts through the utilization of the HPLC/L-MS methodology. [25]

Preparation of Extract

Soxhlet method

The dried leaves of E micrantha were powdered coarsely and extracted using a soxhlet apparatus^[26] at 50°C with methanol and ethanol. Then the rotary evaporator was used to concentrate the extract at a low temperature (40– 60°C) and low pressure after first passing through cotton clothes and Whatman filter paper (No. 1).

Aqueous extraction (Decoction method)

For fifteen minutes, the *F. micrantha* plant sample was cooked in double-distilled water. The liquid was allowed to cool to room temperature for fifteen minutes before being filtered through muslin fabric. To concentrate the extract, the filtered solution was heated one more. The resulting concentrated extract was desiccated and stored in an airtight container, following the procedure outlined.^[27]

The extraction yield for all extracts was determined by employing the subsequent equation:

$$Percentage\ yield = \frac{Actual\ yield}{theoretical\ yield} \times 100$$

Preliminary phytochemical analysis

The extracts of *F. micrantha* leaves were subsequently examined using a preliminary phytochemical method to determine whether different phytoconstituents were present.^[28]

Experimental induction of Hyperlipidemia

Adult male Wistar rats were acclimatized to the laboratory environment for two weeks. A total of six rodents were randomly selected and divided into seven groups. Each group had ad libitum access to food and water throughout the study. The standard and high-fat diets were administered daily to assess the hypolipidemic effect of the treatments. [29,30] The high-fat diet was composed of 42% lipids, 36% carbohydrates, and 22% protein [31]. For *F. micrantha* leaf extract OECD recommendations were followed when conducting the acute toxicity investigation, and no acute toxicity was seen at any of the subsequent choices: 5, 50, 300 and 200 milligrams per kilogram. [13]

Group I (Standard control)

Administered a standard diet and saline/vehicle.

• Group II (High-fat diet control)

Fed a standard pellet diet along with a diet heavy in fat (22% protein, 36% carbohydrate, and 42% fat) procured from Vishnu Traders, Roorkee, Uttarakhand, India for six weeks.

• Group III (Standard drug - atorvastatin)

Following three weeks on the high-fat diet, rats were administered atorvastatin at 20 mg/kg/day for the remaining three weeks while continuing the high-fat diet [32]

• Group IV (FMAQ 200 mg/kg)

After three weeks on the high-fat diet, rats received FMAQ at 200 mg/kg/day for the remaining three weeks while continuing the high-fat diet.

• Group V (FMAQ 400 mg/kg)

After three weeks on the high-fat diet, rats were administered FMAQ at 400 mg/kg/day for the remaining three weeks while continuing the high-fat diet.

• Group VI (FME 200 mg/kg)

After three weeks on the high-fat diet, rats were given FME at 200 mg/kg/day for the remaining three weeks while continuing the high-fat diet.

• Group VII (FME 400 mg/kg)

After three weeks on the high-fat diet, rats received FME at 400 mg/kg/day for the remaining three weeks while continuing the high-fat diet.



Following an overnight fast, the blood serum was extracted from the retro-orbital plexus at the end of the 45th day in order to examine biochemical markers. Serum levels of LDL, HDL, triglycerides, and total cholesterol were estimated.^[33]

Analysis of statistics

The outcomes showed up as mean \pm SD. An analysis of variance (ANOVA) in one direction using the Graph Pad Prism Ver. 7 software and the Bonferroni test were used to determine the significance of the variations among the groupings. The significance was defined as p is less than 0.001.

RESULTS

Organoleptic Evaluations

The leaves of *F. micrantha* are the compound pinnate, up to 10 cm long with 7-13 leaflets (Table 1).

Microscopic Investigation

The transverse section analysis of the F. micrantha leaf revealed essential structural details. Compact parenchyma cells with narrow walls make up the ground tissue. The semicircular vascular strand is composed by parallel radial files of phloem and large, angular, thickwalled xylem components (as depicted in Fig. 1). The stomata on the underside of the leaf are anomocytic in nature (as depicted in Fig. 1). Microscopic examination of the leaf powder showed trichomes with a solitary cell on the epidermis surface (as depicted in Fig. 2A) and discolored starch granules (as depicted in Fig. 2B). Additionally, vessels with indentations (as illustrated in Fig. 2C) and embedded starch granules were identified (as depicted in Fig. 2D), along with xylem vessels, starch granules, and prismatic oxalate crystals (as illustrated in 2E and 2F).

Chemical and Physical Studies

The observations of chemical and physical studies are placed in Table 2.

Table 1: Organoleptic characters of the plant *F. micrantha* Lingelsh

S. No.	Parameters	Leaf
1.	Color	Green
2.	Odor	Characteristic
3.	Taste	Characteristic
4.	Size	length 20-35 cm
5.	Shape	Opposite, pinnately compound, with 7–13 leaflets, elliptic to narrowly elliptic
6.	Touch	Smooth, coarsely serrated margins

Table 2: Physico-chemical analysis *F. micrantha* Lingelsh

Parameters	Percent by weight
Foreign matter	1.170 ± 0.075
Total ash value	15.800 ± 0.1
Acid insoluble ash value	3.333 ± 0.404
Water-soluble ash value	1.033 ± 0.660
Methanol extractive value	17.500 ± 0.871
Alcoholic extractive value	20.430 ± 0.763
Aqueous extractive value	14.433 ± 0.665
Loss on drying	6.993 ± 0.490

Table 3: Pesticide residues (Total organic chloride and total organic phosphorous)

S. No.	Test parameter	Result	Limit (mg/kg)
1.	Alachlor	Not detected	NMT 0.02
2.	Aldrin and dieldrin	Not detected	NMT 0.05
3.	DDT	Not detected	NMT 1.0
4.	Deltamethrin	Not detected	NMT 0.5
5.	Diazinon	Not detected	NMT 0.5
6.	Endosulfan	Not detected	NMT 3.0
7.	Endrin	Not detected	NMT 0.05
8.	Ethion	Not detected	NMT 2.0
9.	Fenitrothion	Not detected	NMT 0.5
10.	Fenvalerate	Not detected	NMT 1.5
11.	Heptachlor	Not detected	NMT 0.05
12.	Hexachlorobenzene	Not detected	NMT 0.1
13.	Lindane	Not detected	NMT 0.6
14.	Malathion	Not detected	NMT 1.0
15.	Parathion	Not detected	NMT 0.5
16.	Phosalone	Not detected	NMT 0.1
17.	Pyrethrins	Not detected	NMT 3.0
18.	Quintozene	Not detected	NMT 1.0

Estimation of Contamination Parameters

Pesticide content

Determining pesticide residues was conducted using the methodologies outlined in the procedure. The following tables, (specifically Table 3), present the outcomes of an in-depth examination of pesticide residues.

The analysis of pesticide residues in the sample reveals that none of the tested pesticides were detected. The results confirm the sample's compliance with the established pesticide residue standards, ensuring its safety for consumption or use.

Heavy metals (Lead, Arsenic, Cadmium and Mercury)

The identification of heavy metals was conducted using the methodologies outlined in the procedure. The presented tables, (namely Table 4), comprehensively depict the

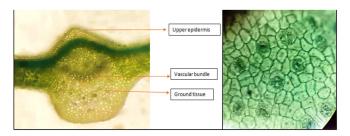


Fig. 1: Microscopic investigation: Transverse section of *Fraxinus* micrantha leaf

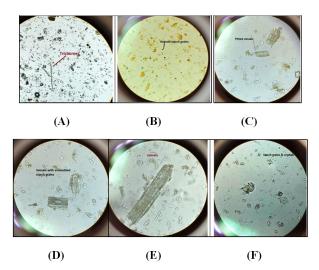


Fig. 2: Powder microscopy of *F. micrantha* leaf (A) Trichomes, (B) Stained starch grain, (C) Pitted vessels, (D) Vessels with embedded starch grain, (E) Vessels (F) Starch grain and crystals

outcomes derived from an in-depth examination of heavy metals residues.

Aflatoxins

The presence of aflatoxins was determined using the methodologies outlined in the procedure. The tables presented, (specifically Table 5), comprehensively depict the outcomes derived from an in-depth examination of aflatoxin residues.

Microbial contamination

The methodologies outlined in the procedure were employed to determine the presence of microbial contamination. (Table 6 presents the outcomes of a comprehensive examination conducted on pesticide residues).

Preliminary phytochemical analysis

The study of phytochemicals in *F. micrantha* leaves signifies the existence of several medicinally significant constituents across different extracts. The presence of flavonoids, alkaloid compounds, coumarins, steroids, triterpenoids and saponins in the methanol extract suggests that it may have an array of medicinal uses. The ethanol extract's pharmacological relevance is further supported by the discovery of carbohydrates, alkaloids, flavonoids, coumarins, and steroids. An aqueous extract includes carbohydrates, flavonoids, coumarins, steroids, and triterpenoids, though it lacks alkaloids and saponins (Table 7).

Table 4: Heavy metals (Lead, Cadmium, Mercury and Arsenic)

S. No.	Test parameter	Result	LOQ (mg/kg)	Limit
1.	Lead	Not detected	0.20	NMT 10ppm
2.	Cadmium	Not detected	0.20	NMT 0.3ppm
3.	Mercury	Not detected	0.20	NMT 1ppm
4.	Arsenic	Not detected	0.20	NMT 3ppm

Table 5: Aflatoxins

S. No.	Test parameter	Result	Limit
1.	B1	Not Detected	NMT 2.0 ppb
2.	B1+B2+G1+G2	Not Detected	NMT 5.0 ppb

Table 6: Microbial contamination

S. No.	Test parameter	Result	Limit
1.	Total viable count	1125cfu/gm	NMT 100000cfu/gm
2.	Total yeast & mould count	94cfu/gm	NMT 1000cfu/gm
3.	E. coli	P*/gm	A**/gm
4.	Salmonella	P*/gm	A**/gm
5.	S. aureus	P*/gm	A**/gm

P*: present, A**: absent



Table 7: Phytochemical composition of *F. micrantha* Lingelsh leaves

S. No.	Class of compounds	Chemical tests	Methanol	Ethanol	Aqueous
1.	Carbohydrate	Molisch	Absent	Present	Present
2.	Reducing sugar	Fehling	Absent	Absent	Absent
	A11 1 + 1	Dragendorff	Present	Present	Absent
3.	Alkaloid	Mayer	Present	Present	Absent
4.	Anthraquinone glycoside	Bontrager	Absent	Present	Absent
5.	Cardiac glycoside	Lieberman-Burchard	Absent	Absent	Absent
6.	Saponin	Froth test	Absent	Absent	Absent
7.	Flavonoid	Lead acetate	Present	Present	Present
8.	Coumarins	Ferric chloride	Present	Present	Present
9.	Cyanogenetic glycoside	Sodium picrate	Absent	Absent	Absent
10.	Steroids and triterpenoids	Liebermann's test	Present	Present	Present

Table 8: Hypolipidemic effect of *F. micrantha* plant extract on high-fat diet-induced Wistar rat model

Group No.	Treatment	Total cholesterol	Triglycerides	HDL	LDL
I	Normal control (Vehicle treated)	122.22 ± 2.751	90.21 ± 0.967	55.81 ± 4.489	35.16 ± 6.473
II	HFD induced only	210.66 ± 2.934	191.57 ± 8.099	16.89 ± 0.667	102.65 ± 3.568
III	HFD+ Standard atorvastatin (20 mg/kg)	103.14 ± 2.631**	92.35 ± 1.723**	49.66 ± 7.142**	25.41 ± 8.523**
IV	HFD+ FMAQ (200mg/kg)	132.74 ± 1.706**	146.54 ± 17.086**	26.25 ± 2.969**	69.20 ± 3.257**
V	HFD+ FMAQ (400mg/kg)	118.81 ± 0.945**	117.35 ± 8.960**	32.96 ± 4.833**	83.27 ± 6.124**
VI	HFD+ FME (200mg/kg)	138.25 ± 2.105**	131.29 ± 3.921**	29.18 ± 7.954**	89.57 ± 8.256**
VII	HFD+ FME (400mg/kg)	112.95 ± 1.706**	112.36 ± 2.230**	35.60 ± 9.246**	51.06 ± 8.239**

One-way ANOVA followed by the Bonferroni test, with values reported as MEAN SD at n=6, *p<0.05, **p<0.001 compared to group HFD treated.

Hypolipidemic Activity

The hypolipidemic activity results demonstrated that both FMAQ and FME extracts effectively increase the HDL along with decrease triglyceride level, LDL, and serum cholesterol (Table 8).

DISCUSSION

The organoleptic analysis of *F. micrantha* Lingelsh leaves reveals distinct characteristics useful for identification. The leaves are green, with a characteristic odor and a typical leaf powder taste. They measure 20 to 35 cm in length, are opposite and are pinnately compound with 7 to 13 elliptic to narrowly elliptic leaflets. The smooth texture with coarsely serrated margins further distinguishes the plant. These features are vital for verifying the plant's authenticity and quality in herbal applications. The transverse section analysis of the *F. micrantha* leaf revealed essential structural details. These anatomical features are crucial for the identification and quality assessment of the plant material.

In essence, standardization is about ensuring that herbal medicines are safe, effective, and reliable for consumers. [34] The physicochemical analysis of F. micrantha Lingelsh provides important insights into its safety. The low foreign

matter content (1.170%) suggests minimal contamination. The total ash value, which represents the total amount of inorganic material, was found to be 15.800%, indicating a moderate level of mineral content. Within this, the acid-insoluble ash, which reflects the amount of siliceous material, was 3.333%, pointing to a small amount of nondigestible material. The water-soluble ash, which helps in determining the presence of water-soluble salts, was observed to be 1.033%, showing that only a small fraction of the total ash is water-soluble. The extractive values in methanol (17.500%), alcohol (20.430%), and aqueous solutions (14.433%) suggest varying levels of bioactive constituents. The loss on drying was measured at 6.993%, which reflects the moisture content in the plant material. This relatively low value suggests that the material is fairly dry, reducing the likelihood of microbial growth and degradation.

The analysis of pesticide residues in the sample reveals that none of the tested pesticides were detected. The results confirm the sample's compliance with the established pesticide residue standards, ensuring its safety for consumption or use. Lead, mercury, cadmium, and arsenic were among the heavy metals that were analyzed, but none of them were found. This means that the sample meets the

safety standards, with heavy metal concentrations well within the acceptable limits, ensuring it is safe for use or consumption. The aflatoxin study suggests that the plant samples are free from detectable levels of aflatoxins and are in compliance with established safety limits for these contaminants. The aflatoxin analysis revealed that neither B1 aflatoxin nor the total aflatoxins were detected in the plant samples. Both results are below the specified limits (2.0 ppb for Aflatoxin B1 and 5.0 ppb for the total aflatoxins), indicating that the plant samples are free from detectable aflatoxin contamination and meet safety standards.

The microbial contamination assessment of the plant sample showed a total viable count of 1125 cfu/g, well within the acceptable limit of 100,000 cfu/g, indicating a low overall microbial load. The total yeast and mold count was 94 cfu/g, significantly below the limit of 1000 cfu/g, suggesting minimal yeast and mold contamination. However, the presence of potentially pathogenic bacteria, including *E. coli, Salmonella*, and *S. aureus*, indicated by "P*/gm," raises concerns as their acceptable limits are zero. This suggests potential contamination that requires further investigation and corrective action to ensure the sample's safety.

The phytochemical analysis of *F. micrantha* leaves indicates the presence of alkaloids, flavonoids, coumarins, steroids, triterpenoids, and saponins in methanol extract, carbohydrates, alkaloids, flavonoids, flavonoids, coumarins, steroids in ethanol extract and carbohydrates, flavonoids, coumarins, steroids, and triterpenoids in aqueous extract. These phytochemicals contribute to the plant's diverse pharmacological activities, potentially offering various therapeutic benefits.

Cholesterol is a vital component of animal life and is essential for the typical operation of every animal cell. It is involved in numerous activities, including the cell membrane, Hormones, bile acids and gene transcription. [35] Cholesterol is synthesized in animal cells through the mevalonate pathway, which is regulated by several mechanisms, including HMG-CoA reductase, its inhibition and hormonal control must use one of two routes—the Kandutsch-Russell pathway or the bloch pathway —to transform the resultant lanosterol into cholesterol. [36] Additionally, it is a primary reason for cardiovascular conditions such as coronary heart disease, atherosclerosis, and myocardial infarction (MI). [37]

The plant has been traditionally used for liver disorders and oxidative stress, which adds a unique angle to its hypolipidemic potential. The study assessed the leaves of *F. micrantha* for their hypolipidemic effects in a high-fat diet-induced Wistar rat model. Rats given a diet high in fat showed a significant rise in blood cholesterol, and triglycerides, along with LDL levels while HDL levels fell. When administered at a dosage of 400 mg per kg, FMAQ and FME extract significantly reduced serum levels of LDL, triglycerides, and cholesterol while increasing HDL levels,

which were close to the recommended atorvastatin dosage of 20 mg/kg. Significant percentage increases in HDL and decreases in triglycerides, LDL, and serum cholesterol in the test extract were also equivalent to those observed with the standard treatment. These findings suggest that both FMAQ and FME extracts of *F. micrantha* exhibit hypolipidemic effects, with FME at higher doses showing more pronounced improvements in lipid profiles compared to FMAQ.

CONCLUSION

The investigation into the hypolipidemic potential of F. micrantha reveals that this plant holds considerable promise as a natural remedy for managing hyperlipidemia. The phytochemical analysis identified key bioactive compounds such as flavonoids, coumarins, and alkaloids across different extracts, contributing to the plant's therapeutic properties. The results from the high-fat diet-induced Wistar rat model demonstrate that both F. micrantha aqueous (FMAO) and methanol extracts (FME) successfully raised HDL levels while decreasing LDL, triglycerides, and total cholesterol to enhance lipid profiles. Notably, FME showed more pronounced effects compared to FMAQ, indicating its greater potential as a hypolipidemic agent. These results imply that *F. micrantha* may be a useful natural substitute for traditional synthetic hypolipidemic medications, offering a safer and more costeffective option in the treatment of heart diseases due to obesity for the long-term management of hyperlipidemia. Further research, including clinical trials, is warranted to fully establish the efficacy and safety of F. micrantha for therapeutic use.

REFERENCES

- Hill MF, Bordoni B. Hyperlipidemia. In: StatPearls. StatPearls Publishing, Treasure Island (FL); 2023. PMID: 32644608.
- 2. Bahmani M, Mirhoseini M, Shirzad H, Sedighi M, Shahinfard N, Rafieian-Kopaei M. A Review on Promising Natural Agents Effective on Hyperlipidemia. Journal of Evidence-Based Complementary & Alternative Medicine. 2015;20(3):228-238. Available from: https://doi:10.1177/2156587214568457
- 3. Dalwadi PP, Patani PV. Anti hyperlipidemic activity of Tephrosia purpurea plant extracts in poloxomer 407 induced hyperlipidemic rats. International Journal of Pharmacological Research. 2014;4(4):186-93. Available from: http://dx.doi.org/10.7439/ijpr. y4i4.122
- Sheeba DA, Gandhimathi R. An Overview on Hyperlipidemia. Journal of Pharmaceutical Research International. 2021 Dec 18;33(59B):543-55. Available from: https://doi.org/10.9734/ jpri/2021/v33i59B34413
- Ford I, Murray H, McCowan C, Packard CJ. Long-term safety and efficacy of lowering low-density lipoprotein cholesterol with statin therapy: 20-year follow-up of West of Scotland Coronary Prevention Study. Circulation. 2016;133(11):1073-80. Available from: https:// doi.org/10.1161/CIRCULATIONAHA.115.019014
- Alagumanivasagam, G., & Veeramani, P. A review on medicinal plants with hypolipidemic activity. International Journal of Pharmacy and Analytical Research. 2015; 4(2):129-34. Available from: https://doi.org/10.61096/ijpar.v4.iss2.2015.129-134
- 7. Wannasiri S, Piyabhan P, Naowaboot J. Rhinacanthus nasutus leaf improves metabolic abnormalities in high-fat diet-induced



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- obese mice. Asian Pacific Journal of Tropical Biomedicine. 2016 Jan 1;6(1):1-7. Available from: https://doi.org/10.1016/j.apitb.2015.10.004
- Wallander E. Systematics of Fraxinus (Oleaceae) and evolution of dioecy. Plant Systematics and Evolution. 2008 Jun;273(1):25-49. Available from: https://doi.org/10.1007/s00606-008-0005-3
- Kumar P, Sati SC. Chemical composition, antioxidant and antimicrobial activities of Himalayan Fraxinus micrantha Lingelsh leaf extract. Natural Product Research [Internet]. 2021;35(20):3519-3523. Available from: https://doi.org/10.1080/ 14786419.2019.1710706
- 10. Garbyal SS, Grover A, Agrawal KK, Babu CR. Traditional phytomedicinal knowledge of Bhotias of Dharchula in Pithoragarh. Indian Journal of traditional knowledge. 2007;6(2):360-364. Available from: https://www.researchgate.net/publication/285978179_Traditional_phytomedicinal_knowledge_of_Bhotias_of_Dharchula_in_Pithoragarh
- 11. Sarfraz I, Rasul A, Jabeen F, Younis T, Zahoor MK, Arshad M, Ali M. Fraxinus: A Plant with Versatile Pharmacological and Biological Activities. Evid Based Complement Alternat Med. 2017;2017(1):4269868. Available from: https://doi.org/10.1155/2017/4269868
- 12. Iqbal PF, Parveen H, Bhat AR, Azam A. IN VITRO SUSCEPTIBILITY OF ENTAMOEBA HISTOLYTICA TO FRAXINUS MICRANTHA LEAF EXTRACTS AND ITS ISOLATED CONSTITUENTS (TRITERPENES AND COUMARINS). World. J. Pharma Res. 2015 Apr 29;4:1255-69. Available from: https://www.semanticscholar.org/paper/IN-VITRO-SUSCEPTIBILITY-OF-ENTAMOEBA-HISTOLYTICA-TO-Iqbal-Parveen/7878c503260c611e422fd566db5298e94977b1da
- 13. Srivastava S, Virmani T, Haque MR, Alhalmi A, Al Kamaly O, Alshawwa SZ, Nasr FA. Extraction, HPTLC analysis and antiobesity activity of Jatropha tanjorensis and Fraxinus micrantha on high-fat diet model in rats. Life. 2023 May 25;13(6):1248. Available from: Available from: https://doi.org/10.3390/life13061248
- 14. Umamaheshwari D, Muthuraja R, Kumar M, Venkateswarlu BS. Standardization of Herbal Drugs A Overview. International Journal of Pharmaceutical Sciences Review and Research. 2021;68(1):213-219. Available from: http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.033
- 15. Singh B. Pharmacognostic Standardizations of Traditionally used Hepatoprotective Plant Fraxinus micrantha. International Journal of Green Pharmacy (IJGP). 2018 Aug 12;12(02). Available from: https://doi.org/10.22377/ijgp.v12i02.1827
- 16. Schweingruber FH, Börner A, Schulze ED. Atlas of Stem Anatomy in Herbs, Shrubs and Trees: Volume 1. Springer Science & Business Media; 2011 Mar 18. Available from: https://link.springer.com/ book/10.1007/978-3-642-11638-4
- 17. Johansen DA. Plant Microtechnique. New York: Mc-Graw-Hillbook Company.
- 18. Naheed S, Hussain K, Shehzadi N, Saeed H, Parveen S, Ali E, Shaukat A, Saghir F, Iftikhar S, Khan MT. Microscopic, phytochemical and antibacterial studies of Zizyphus oxyphylla Edgew. leaves. Pakistan Journal of Pharmaceutical Sciences. 2022 Mar 2;35. Available from: https://doi.org/10.36721/PJPS.2022.35.2.SP.685-694.1
- 19. Ayurvedic Pharmacopoeia Committee. The ayurvedic pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare. New Delhi, India: Department of AYUSH. 2001;1(1):144-5.
- 20. Ghosh D, Mondal S, Ramakrishna K. Pharmacobotanical, physicochemical and phytochemical characterisation of a rare salt-secreting mangrove Aegialitis rotundifolia Roxb., (Plumbaginaceae) leaves: A comprehensive pharmacognostical study. South African Journal of Botany. 2017 Nov 1;113:212-29. Available from: https://doi.org/10.1016/j.sajb.2017.08.012
- 21. World Health Organization. Quality control methods for herbal materials. World Health Organization; 2011.
- 22. Ambrus A, Füzesi I, Susan M, Dobi D, Lantos J, Zakar F, Korsos I, Olah J, Beke BB, Katavics L. A cost-effective screening method for

- pesticide residue analysis in fruits, vegetables, and cereal grains. Journal of Environmental Science and Health. 2005 Mar 1;40(2):297-339. Available from: https://doi.org/10.1081/PFC-200045554
- 23. Bauer R. Quality criteria and standardization of phytopharmaceuticals: Can acceptable drug standards be achieved? Ther Innov Regul Sci. 1998; 32(1):101-110. Available from: https://doi.org/10.1177/009286159803200114
- 24. Luo L, Wang B, Jiang J, Fitzgerald M, Huang Q. Heavy metal contaminations in herbal medicines: Determination, comprehensive risk assessments, and solutions. Frontiers in pharmacology. 2021;11:595335. Available from: https://doi.org/10.3389/ fphar.2020.595335
- 25. Joshi VK, Joshi A, Dhiman KS. The Ayurvedic Pharmacopoeia of India, development and perspectives. Journal of Ethnopharmacology. 2017;197:32-38. Available from: https://doi.org/10.1016/j. jep.2016.07.030
- 26. Kokate CK. Practical pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 1993.
- 27. Martins N, Barros L, Santos-Buelga C, Henriques M, Silva S, Ferreira IC. Evaluation of bioactive properties and phenolic compounds in different extracts prepared from Salvia officinalis L. Food Chemistry. 2015;170:378-385. Available from: https://doi.org/10.1016/j.foodchem.2014.08.096
- 28. Viswanathan MB, Ananthi JJ, Venkateshan N. Pharmacognostical Studies on the leaves of Jatropha tanjorensis. Research Journal of Pharmacognosy and Phytochemistry. 2018;10(4):291-8. Available from: https://doi.org/10.5958/0975-4385.2018.00047.X
- 29. Parab RS, Mengi SA. Hypolipidemic activity of Acorus calamus L. in rats. Fitoterapia 2002;73(6):451. Available from: https://doi.org/10.1016/s0367-326x(02)00174-0
- 30. Sripradha R, Sridhar MG, Maithilikarpagaselvi N. Antihyperlipidemic and antioxidant activities of the ethanolic extract of Garcinia cambogia on high fat diet-fed rats. Journal of Complementary and Integrative Medicine. 2016 Mar 1;13(1):9-16. Available from: https://doi.org/10.1515/jcim-2015-0020
- 31. Haque MR, Ansari SH, Najmi AK. Cuminum cyminum L. fruits distillate ameliorates the high fat diet-induced obesity. Pharmacognosy Communications 2013;3(4):49-57. Available from: https://doi.org/10.5530/pc.2013.4.7
- 32. T Mahesh Babu Et Al: Standardization Of Herbal Drugs A Review. International Ayurvedic Medical Journal. 2017;5(1):269-277. Available from: http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.033
- 33. Abid R, Mahmood R, Santosh Kumar HS. Hypolipidemic and antioxidant effects of ethanol extract of Cassia fistula fruit in hyperlipidemic mice. Pharmaceutical biology. 2016 Dec 1;54(12):2822-9. Available from: https://doi.org/10.1080/13880 209.2016.1185445
- 34. Ray AS, Rahaman CH. Pharmacognostic standardization and phytochemical investigation of Cajanus scarabaeoides (L.) Thouars. Research Journal of Pharmacognosy and Phytochemistry. 2018;10(1):120-31. Available from: http://dx.doi.org/10.5958/0975-4385.2018.00018.3
- 35. Lehninger AL, Nelson DL, Cox MM Lehninger. Principles of Biochemistry. New York, USA: Worth Publishers; 2000. Available from: http://dx.doi.org/10.1007/978-3-662-08289-8
- 36. Singh P, Saxena R, Srinivas G, Pande G, Chattopadhyay A. Cholesterol biosynthesis and homeostasis in regulation of the cell cycle. PloS one. 2013 Mar 15;8(3):e58833. Available from: https://doi.org/10.1371/journal.pone.0058833
- 37. Ighodaro OM, Omole JO. Effects of Nigerian Piliostigma thonningii species leaf extract on lipid profile in Wistar rats. International Scholarly Research Notices. 2012;2012(1):387942. Available from: https://doi.org/10.5402/2012/387942
- 38. Singh H, Kaur S, Arora S, Singh B. Ameliorative potential of a traditionally used plant *Fraxinus micrantha* against oxidative stress and paracetamol-induced hepatotoxicity. Pharmacognosy Magazine. 2019;15(66):S433-9. Available from: https://doi.org/10.4103/pm.pm_236_19

HOW TO CITE THIS ARTICLE: Srivastava S, Singh Y, Garg A, Upadhayay A, Haque R, Virmani T. Standardization and Hypolipidemic Effect of *Fraxinus micrantha* Leaf. Int. J. Pharm. Sci. Drug Res. 2025;17(2):144-151. **DOI**: 10.25004/IJPSDR.2025.170204