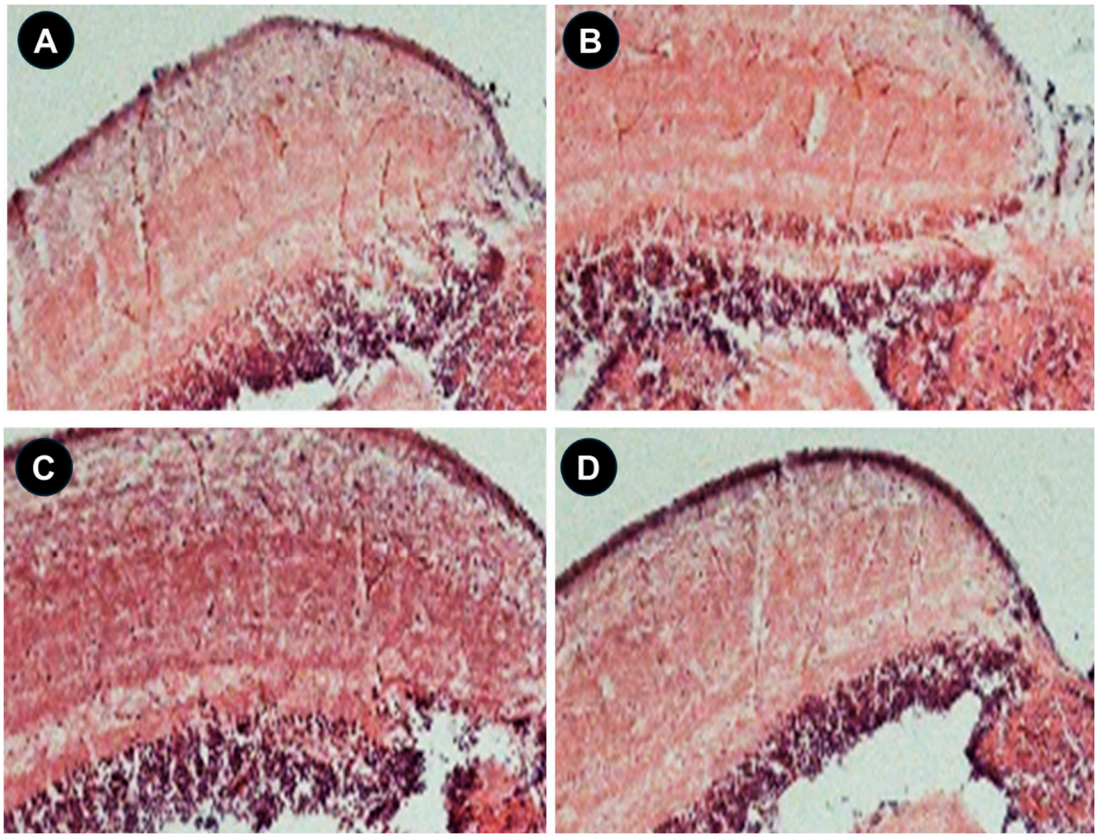
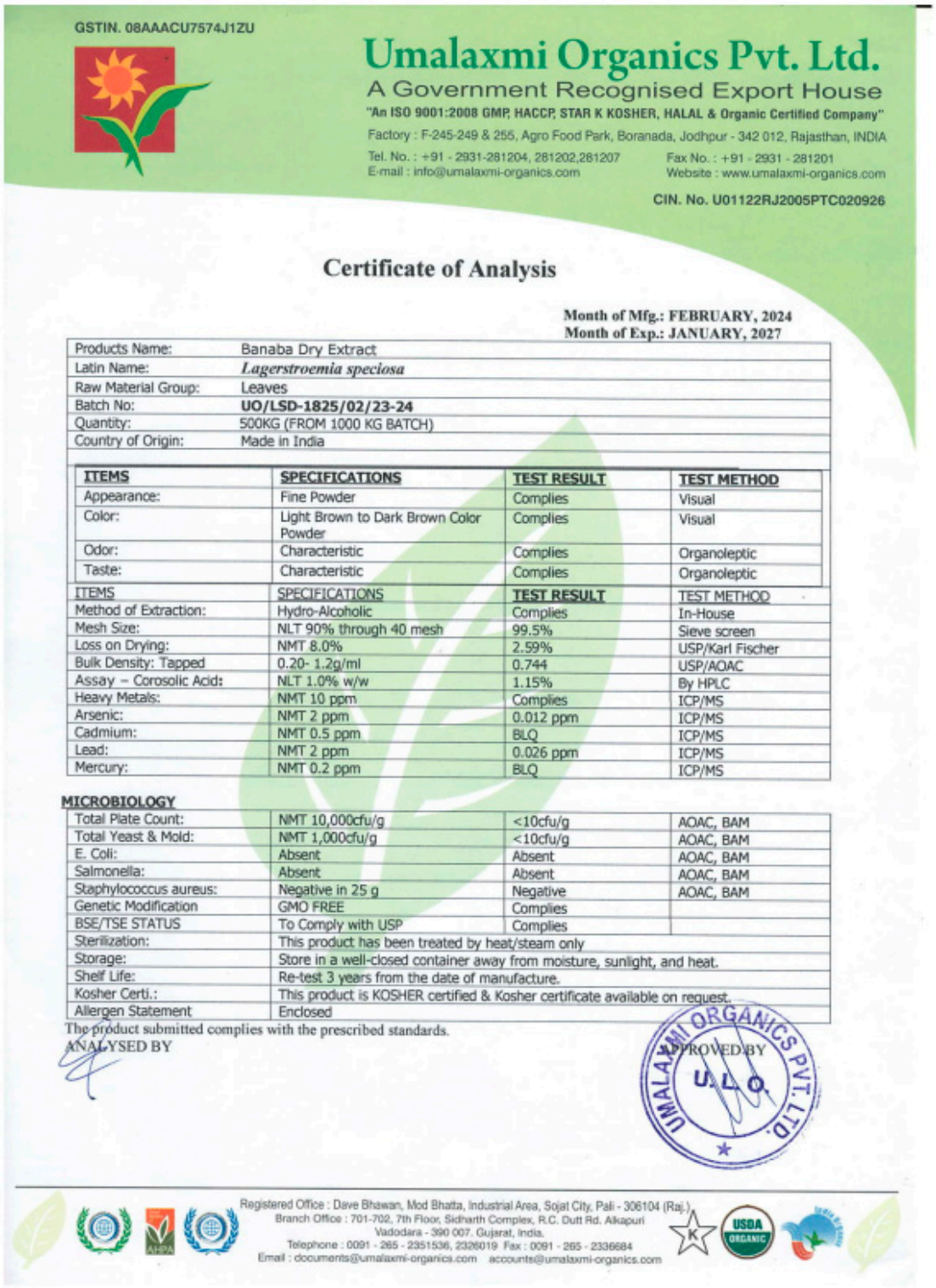
Supplementary Material

Supplementary Figure S1



Supplementary Figure S1. A digitally magnified view of the H&E brain section (figure 9B, covered with the dotted black box). (A), (B), (C) and (D) represent the magnified view of the brain section HC+HG, BLE, POL and BLE+POL supplemented group, respectively. The HC+HG indicates high cholesterol (4% w/w, final) + high galactose (30% w/w, final) diet. BLE, POL and BLE+POL indicating banaba leaf extract (0.1% w/w, final), policosanol (0.1% w/w, final) and banaba leaf extract +policosanol mixed preparation (0.1% w/w each) amalgamated with HC+HG.

Supplementary Table S1



GSTIN. 08AAACU7574J1ZU

Umalaxmi Organics Pvt. Ltd.

A Government Recognised Export House

"An ISO 9001:2008 GMP, HACCP, STAR K KOSHER, HALAL & Organic Certified Company"

Factory : F-245-249 & 255, Agro Food Park, Boranada, Jodhpur - 342 012, Rajasthan, INDIA

Tel. No. : +91 - 2931-281204, 281202,281207 Fax No. : +91 - 2931 - 281201

E-mail : info@umalaxmi-organics.com Website : www.umalaxmi-organics.com

CIN. No. U01122RJ2005PTC020926

Certificate of Analysis

Month of Mfg.: FEBRUARY, 2024
Month of Exp.: JANUARY, 2027

Products Name:	Banaba Dry Extract
Latin Name:	*Lagerstroemia speciosa*
Raw Material Group:	Leaves
Batch No:	**UO/LSD-1825/02/23-24**
Quantity:	500KG (FROM 1000 KG BATCH)
Country of Origin:	Made in India

ITEMS	SPECIFICATIONS	TEST RESULT	TEST METHOD
Appearance:	Fine Powder	Complies	Visual
Color:	Light Brown to Dark Brown Color Powder	Complies	Visual
Odor:	Characteristic	Complies	Organoleptic
Taste:	Characteristic	Complies	Organoleptic
ITEMS	SPECIFICATIONS	TEST RESULT	TEST METHOD
Method of Extraction:	Hydro-Alcoholic	Complies	In-House
Mesh Size:	NLT 90% through 40 mesh	99.5%	Sieve screen
Loss on Drying:	NMT 8.0%	2.59%	USP/Karl Fischer
Bulk Density: Tapped	0.20- 1.2g/ml	0.744	USP/AOAC
Assay – Corosolic Acid:	NLT 1.0% w/w	1.15%	By HPLC
Heavy Metals:	NMT 10 ppm	Complies	ICP/MS
Arsenic:	NMT 2 ppm	0.012 ppm	ICP/MS
Cadmium:	NMT 0.5 ppm	BLQ	ICP/MS
Lead:	NMT 2 ppm	0.026 ppm	ICP/MS
Mercury:	NMT 0.2 ppm	BLQ	ICP/MS

MICROBIOLOGY

Total Plate Count:	NMT 10,000cfu/g	<10cfu/g	AOAC, BAM
Total Yeast & Mold:	NMT 1,000cfu/g	<10cfu/g	AOAC, BAM
E. Coli:	Absent	Absent	AOAC, BAM
Salmonella:	Absent	Absent	AOAC, BAM
Staphylococcus aureus:	Negative in 25 g	Negative	AOAC, BAM
Genetic Modification	GMO FREE	Complies	
BSE/TSE STATUS	To Comply with USP	Complies	
Sterilization:	This product has been treated by heat/steam only		
Storage:	Store in a well-closed container away from moisture, sunlight, and heat.		
Shelf Life:	Re-test 3 years from the date of manufacture.		
Kosher Certi.:	This product is KOSHER certified & Kosher certificate available on request.		
Allergen Statement	Enclosed		

The product submitted complies with the prescribed standards.

ANALYSED BY

APPROVED BY

UMALAXMI ORGANICS PVT. LTD. U. L. O.

Registered Office : Dave Bhawan, Mod Bhatta, Industrial Area, Sojat City, Pali - 306104 (Raj.)
Branch Office : 701-702, 7th Floor, Sidharth Complex, R.C. Dutt Rd. Alkapuri
Vadodara - 390 007. Gujarat, India.
Telephone : 0091 - 265 - 2351536, 2326019 Fax : 0091 - 265 - 2336684
Email : documents@umalaxmi-organics.com accounts@umalaxmi-organics.com

Supplementary Table S1: A certificate of analysis of the used banaba leaf extract (BLE).

Supplementary Table S2

RAYDEL
Health Confidence

Raydel Australia Pty Ltd
Level 1, building 1, 9-15 Chilvers Rd.
Thornleigh NSW 2120 Australia
Tel +61 2 9480 1300
Fax +61 2 9480 1399
A.B.N. 45 054 555 903
www.raydel.com.au
info@raydel.com.au

Product name: Policosanol

Batch #: 310030324

Date of Manufacture: 12/03/2024

Parameter	Results	Approved Limits
Color	Complies	Off white to cream
Identity and Purity*		
1-tetracosanol (C_{24})	0.05 %	0.00 – 2.0 %
1-hexacosanol (C_{26})	3.34 %	3.0 –10.0 %
1-heptacosanol (C_{27})	0.82 %	0.1 - 3.0 %
1-octacosanol (C_{28})	60.02 %	60.0 –70.0 %
1-nonacosanol (C_{29})	0.55 %	0.1- 2.0 %
1-triacontanol (C_{30})	14.96 %	10.0 – 15.0%
1-dotriacontanol (C_{32})	8.24 %	5.0 - 10.0 %
1-tetratriacontanol (C_{34})	2.39 %	0.1 – 5.0 %
Total (Purity*)	**90.37 %**	**≥ 90 %**
Other quality specifications		
Melting temperature	81.3-83.0 °C	78.0 – 83.0 °C
Loss on drying	0.54 %	≤ 1.0 %
Residue of ignition	0.72 %	≤ 0.85 %
Heavy metals (Pb, Cd, Hg)	<0.000015 %	≤ 0.001 %
Sodium content	86.34 ppm	≤ 100 ppm
Potassium content	2905.41 ppm	≤ 4500 ppm
Residual solvents		
Acetone	≤ 0.03	≤ 0.03 g/kg
Hexane	≤ 0.005	≤ 0.005 g/kg
Microbiological content		
Total Aerobic Microbial Count	≤10	≤10^3 per g
Yeast and mould	≤10	≤10^2 per g
Enterobacteria or Coliform count	≤10	≤10^2 per g
***Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia* coli, Candida albicans**	Absent	Absent in 1 g
Salmonella sp	Absent	Absent in 10 g
Observations:		
References:	* Manufacturer GC validated method, purity expressed as the total of high molecular weight alcohols	

Not about storage conditions: No special storage conditions are required. The substance hasa self life of 5 years stored under ambient conditions of climatic Zones IV or II, as demonstrated in stabilities studies performed accordingly to ICH guidlines.

Approved (_X_) Released (_X_) Rejected (___)

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Table S2: Certificate of analysis and the composition of the used policosanol (POL).

Supplementary Table S3. Compositing of different formulated diets

Group	HC + HG	HC + HG + BLE	HC + HG + POL	HC + HG BLE +POL
Tetrabits *	6.60	6.59	6.59	6.58
Cholesterol (final 4%, w/w)	0.40	0.40	0.40	0.40
Galactose (final 30%, w/w)	3.00	3.00	3.00	3.00
Policosanol (final 0.1%, w/w)	0.00	0.01	0.00	0.01
Banaba leaf extract (final 4%, w/w)	0.00	0.00	0.01	0.01
Total mixture (mg)	10.00	10.00	10.00	10.00

*Normal tetrabits flake [Tetrabit Gmbh D49304, Melle, Germany a normal zebrafish diet containing 47.5% crude protein, 6.5% crude fat, 2.0% crude fiber, 10.5% crude ash, containing vitamin A (29,770 IU/kg), vitamin D3 (1,860 IU/kg), vitamin E (200 mg/kg), and vitamin C (137 mg/kg)].
BLE, Banaba leaf extract; HC, high cholesterol; HG, high galactose; POL, policosanol.

Section S1. List of the used chemicals: Dihydroethidium (DHE, 104821-25-2, Cat #37291), and acridine orange (AO, 65-61-2, Cat#A9231), oil red O (Cat#O0625), and 2-phenoxyethanol (Sigma P1126; St. Louis, MO, USA), paraoxon-ethyl (Cat. No. 36186) and 5-bromo-4-chloro-3-indolyl β D-galactopyranoside (X-gal, Cat#B54252) were procured from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals and reagents else otherwise stated were of analytical grade and used as supplied.

Section S2. Quantification of blood lipoprotein profile hepatic function biomarkers AST and ALT

The plasma total cholesterol (TC) and triglycerides (TGs) were determined using commercial assay kits (cholesterol, T-CHO, and TGs, Cleantech TS-S; Wako Pure Chemical, Osaka, Japan) as per the method suggested by the suppliers. In brief, 5 μL serum was mixed with 200 μL reaction mixture (supplied with a commercial assay kit) for the TC analysis. The content was incubated at 37°C for 10 min, resulting in a red-colored product quantified by adsorption at 490 nm (Microplate reader, Bio-Rad, Hercules, CA, USA).

Similarly, 5 μL serum was mixed with a 200 μL of TGs-specific reaction mixture (supplied with a commercial assay kit) for TGs analysis. The content was incubated for 10 min at 37°C, and the formed colored product was quantified by taking adsorption at 490 nm.

For HDL-C analysis, serum was mixed in an equal ratio with the separation solution (supplied with a commercial assay kit), followed by centrifugation at 3,000 rpm for 10 min. The supernatant (20 μL) was collected and blended with a 200 μL reaction mixture (supplied with a commercial assay kit). After 10 min incubation at 37°C, red color intensity corresponding to HDL-C was quantified by taking absorption at 490 nm.

The commercial diagnostic kit (Asan Pharmaceutical, Hwasung, Republic of Korea) was used to quantify aspartate transaminase (AST) and alanine transaminase (ALT) levels in the plasma, following the instructions suggested by the manufacturers. Briefly, 5 μL of plasma was combined with 250 μL of either AST or ALT-specific solution, as supplied in the diagnostic kit. Following a 30 min incubation for AST or 60 min incubation of ALT at 37°C, the mixture was then blended with 250 μL of the respective coloring reagent (AST or ATL-specific, provided in the diagnostic kit). After a subsequent 20 min incubation at room temperature, 250 μL of 0.4 N NaOH was introduced to halt the reaction. Finally, the AST and ATL were quantified by measuring absorbance at 490 nm.