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Safety of an aqueous ethanolic extract of *Labisia pumila* as a novel food pursuant to Regulation (EU) 2015/2283

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Abstract

Following a request from the European Commission, the EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) was asked to deliver an opinion on aqueous extract of *Labisia pumila* as a novel food (NF) pursuant to Regulation (EU) 2015/2283. The NF is a standardised hydroalcoholic extract from a dried whole plant (including roots) of *L. pumila*, mixed with maltodextrin (as a drying aid), and proposed by the applicant to be used as a food supplement in amounts up to 750 mg/day. The target population is the general adult population, except pregnant and lactating women. The major constituents of this NF are carbohydrates (up to 85.5%), with a smaller amount of proteins (up to 6.5%), gallic acid (up to 3.7%) and fats (up to 1.6%). The Panel considers that taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous. The provided genotoxicity studies do not raise concerns about the genotoxicity of the NF. Based on the available toxicological data, the Panel considers an intake of up to 5 mg/kg body weight per day as safe. For the target population, this level corresponds to 350 mg/day, which is lower than the use level proposed by the applicant. The Panel concludes that the NF is safe for the target population up to 350 mg/day.

Keywords: aqueous ethanolic extract of *Labisia pumila*, novel food, safety, food supplement

Requestor: European Commission

Question number: EFSA-Q-2019-00694

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

On 7 October 2019, the company Orchid Life Sdn Bhd[1] submitted a request to the European Commission in accordance with Article 10 of Regulation (EU) 2015/22831 to authorise placing on the market of an aqueous extract of *Labisia pumila* as a novel food.

The application requests to authorise use of an aqueous extract of *Labisia pumila* in food supplements for the general adult population, excluding pregnant and lactating women.

The applicant has also requested data protection under Article 26 of Regulation (EU) 2015/2283.

2. Data and Methodologies

2.1. Data

The safety assessment of this NF is based on data supplied in the application, information submitted by the applicant following EFSA requests for supplementary information and additional data identified by the Panel.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in the Commission Implementing Regulation (EU) 2017/2469[2].

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of a NF application (EFSA NDA Panel, 2016). As indicated in this guidance, it is the duty of the applicant to provide all of the available (proprietary, confidential and published) scientific data, (including both data in favour and not in favour) that are pertinent to the safety of the NF.

This NF application includes a request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283. The data requested by the applicant to be protected comprise data on the solubility of the NF and toxicological information (pharmacokinetics, genotoxicity, subchronic and chronic oral toxicity).

2.2. Methodologies

The assessment follows the methodology set out in the EFSA guidance on NF applications (EFSA NDA Panel, 2016) and the principles described in the relevant existing guidance documents from the EFSA Scientific Committee. The legal provisions for the assessment are laid down in Article 11 of Regulation (EU) 2015/2283 and in Article 7 of the Commission Implementing Regulation (EU) 2017/2469.

Additional information, which was not included in the application, was retrieved by literature search, following a search strategy and standard operating procedure as described by Dibusz and Vejvodova (2020).

This assessment concerns only the risks that might be associated with consumption of the NF under the proposed conditions of use, and is not an assessment of the efficacy of the NF with regard to any claimed benefit.

3. Assessment

3.1. Introduction

The NF, which is the subject of the application, is an aqueous ethanolic extract (1:1) of *L. pumila*, mixed with maltodextrin (2:1) as a drying aid ('encapsulation agent'), denominated as SKF7™.

The NF falls under Regulation 2015/2283, Article 3(2)(a)(iv): food consisting of, isolated from or produced from plants or their parts.

The NF is proposed by the applicant to be used as a food supplement for the general adult population, except pregnant and lactating women.

[1] On 26/2/2021, the applicant informed EFSA about the change in the name of the company to Medika Natura Sdn. Bhd.

[2] Commission Implementing Regulation (EU) 2017/2469 of 20 December 2017 laying down administrative and scientific requirements for applications referred to in Article 10 of Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. OJ L 351, 30.12.2017, pp. 64–71

3.2. Identity of the NF

The NF is standardised hydroalcoholic extract from a dried whole plant (including roots) of *L. pumila* and mixed with maltodextrin (as a drying aid).

According to WFO (World Flora Online), the full taxonomy of *L. pumila* is the following:

- Kingdom: Plantae,
- Clade: Angiosperms,
- Order: Ericales Bercht. & J. Presl,
- Family: Primulaceae Batsch ex Borkh (synonym: Myrsinaceae R.Br.),
- Genus: *Labisia* Lindl,
- Species: *Labisia pumila* (Blume) Fern.-Vill.

The plant is also known as *Ardisia pumila* Blume (POWO; WFO, online), *Ardisia malouiana* (L. Linden & Rodigas) Markgr., *Ardisia spicata* Wall. ex A.DC., *Ardisia malouiana* (L. Linden & Rodigas), *Labisia pothoina* Lindl. and *Labisia punctata* (Reinw.) Airy Shaw (WFO, online).

The common names used for this plant are: 'Kacip Fatimah', 'Selusoh Fatimah', 'Pokok pinggang', 'Rumput palis', 'Tadah matahari', 'Mata pelanduk rimba', 'Bunga belungkas hutan', 'Remoyan batu' or 'Sangkoh', according to the applicant.

The plant *L. pumila* is native to Borneo, Cambodia, Java, Malaya, New Guinea, Sumatra, Thailand and West Malaysia (POWO; WFO, online).

To confirm that the plant used in the preparation of the NF belongs to the species *L. pumila* (Blume) Fern.-Vill., the applicant provided an identification report issued by the Forest Research Institute Malaysia (FRIM) which performed a microscopic examination of the provided plant samples together with the BLAST analysis (DNA sequences of *rbc*L chloroplast region).

3.3. Production process

According to the information provided, the NF is produced in a manufacturing facility in Malaysia which is registered with the US FDA. The manufacturing process has been certified under ISO 22000:2005 and conforms to the requirement of Good Manufacturing Practices (GMP).

The plant *L. pumila*, used as a raw material for the production of the NF, is not genetically modified and has been cultivated by the Department of Agriculture at Parit Botak (Malaysia) and according to Malaysia Good Agricultural Practices (MyGAP). No pesticides, antimicrobial or antiparasitic agents are used in the plant cultivation, as stated by the applicant.

After control of raw material, the whole plant is washed, dried and ground. The ground plant is then extracted twice with a mixture of water and ethanol (50/50 v/v). The liquid extract is then concentrated, mixed with maltodextrin (which is used as a drying aid) in a ratio of 2:1 and spray-dried.

The Panel considers that the production process is sufficiently described and does not raise safety concerns.

3.4. Compositional data

The NF mainly consists of carbohydrates (up to 85.5%), with a smaller amount of proteins (up to 6.5%) and fats (up to 1.6%). According to the applicant, the key characteristic component of the NF is gallic acid, which represents up to 3.7% of the NF. Gallic acid was chosen as a key component in accordance with the Malaysian Herbal Monograph and Malaysian Standard (MS 2540:2013).

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with certain characteristics, the applicant provided analytical information on what has been considered key parameters for five independently produced batches of the NF (Table 1). Information was provided on the accreditation of the laboratories that conducted the analyses presented in the application.

Table 1: Batch-to-batch analysis of the NF

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Gallic acid (%, SD)	3.48 ± 0.11	3.65 ± 0.07	3.35 ± 0.02	3.47 ± 0.08	3.71 ± 0.02	HPLC-UV/ViS (In-house)
Mesh size (% through 120 mesh)	94	95	96	100	99	Laser kdiffraction (In-house)
Moisture (%)	5	5	3.5	4.8	5.1	Thermogravimetry (In-house method)
Loss on drying (%)	3.6	2.7	3.8	3.9	5.8	Oven method
Ash (g/100 g)	6.6	6.6	6.3	6.3	6.8	Gravimetry based on AOAC 923.03, 930.30, 940.26, 920.153, 920.155, 950.14, 930.05, 938.08 (In-house)
Acid-insoluble ash (%)	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3	Gravimetry (AOAC 975.12)
Ethanol (%)	0.31	0.33	0.10	0.13	0.73	Gas chromatography (In- house)
Proximate analysis						
Protein (g/100 g)	6.3	6.3	6.5	4.3	NR	Kjeldahl method based on AACC 46-12, Vol. II 9th ed (In-house)
Total fat (g/100 g)	1.6	0.3	0.5	0.8	NR	Gravimetry (AOAC 950.54)
Carbohydrate (g/100 g)	77.8	83.1	82.9	85.5	NR	Calculation (Method of Analysis for Nutrition Labeling, AOAC, 1993)
Sugars (g/100 g)[(a)]	6.7	< 0.3	< 0.3	3.7	NR	Titrimetric5 + 6.6 + based on AOAC 974.06 and 925.05 (In-house)
Dietary fibre (g/100 g)	4.6	5.4	4.1	1.6	NR	Enzymatic-Gravimetry (AOAC 985.29)
Calories (kcal/100 g)	351	360	362	346	367	Calculation (Method of Analysis for Nutrition Labeling, AOAC, 1993)
Contaminants						
Arsenic (mg/kg)	0.18	< 0.0005	0.04	0.003	< 0.0005	AAS based on AOAC 999.10 (In-house)
Cadmium (mg/kg)	0.06	< 0.0005	< 0.0005	< 0.0005	< 0.0005	AAS based on AOAC 999.10 (In-house)
Mercury (mg/kg)	0.019	0.02	0.017	< 0.0001	< 0.0001	AAS (In-house)
Lead (mg/kg)	1.16	< 0.0005	0.6	0.06	< 0.0005	AAS based on AOAC 999.10 (In-house)
Alfatoxin B_1 (μg/kg)	< 1	< 1	< 1	< 1	< 1	HPLC (UVE reactor/FD)
Alfatoxin B_2 (μg/kg)	< 1	< 1	< 1	< 1	< 1	
Alfatoxin G_1 (μg/kg)	< 1	< 1	< 1	< 1	< 1	
Alfatoxin G_2 (μg/kg)	< 1	< 1	< 1	< 1	< 1	

Parameter (unit)	Batch number					Method of analysis
	#1	#2	#3	#4	#5	
Microbiological						
Total plate count (CFU/g)	430	< 10	< 10	150	< 10	British Pharmacopoeia 2016: Appendix XVI B – Pour-plate method
Yeast and mould (CFU/g)	< 10	< 10	< 10	< 10	< 10	
Escherichia coli (in 1 g)	Absent	Absent	Absent	Absent	Absent	British Pharmacopoeia 2016: Appendix XVI F
Staphylococcus aureus (in 1 g)	Absent	Absent	Absent	Absent	Absent	
Salmonella (in 25 g)	Absent	Absent	Absent	Absent	Absent	
Pseudomonas aeruginosa (in 1 g)	Absent	Absent	Absent	Absent	Absent	

HPLC-UV/ViS: high-performance liquid chromatography with ultraviolet/visible light detector; AOAC: Association of Official Agricultural Chemists; AACC: American Association of Cereal Chemists; AAS: atomic absorption spectrophotometry; FD: fluorescence detection; CFU: colony forming units; NR: not reported; SD: standard deviation.
(a): Upon a request to clarify the wide range reported for parameter 'Sugars', the applicant conducted analyses on additional five batches by using HPLC method (AOAC 980.13, AOAC 2018.16). The results ranged from 6.34 g/100 g to 8.24 g/100 g.

In addition to the contaminants listed in Table 1, the applicant also provided the certificates of analyses (CoAs) for dioxins and polychlorinated biphenyls [(PCBs)/dioxins], polycyclic aromatic hydrocarbons (PAHs) and pesticides for the same five batches of the NF. The results showed that analysed PAHs and pesticides levels are below the limit of detection (LOD) or limit of quantification (LOQ) of the analytical methods used.[3] PCBs/dioxins concentrations were found to be on average 0.0422 pg/g wet weight WHO-PCB-TEQ, 0.1832 pg/g wet weight WHO-PCDD/F-TEQ and 0.2256 pg/g wet weight WHO-PCDD/F-PCB-TEQ (upper bound), and do not present safety concerns.

Upon a request, the applicant analysed seven batches of the NF for the presence of pyrrolizidine alkaloids (PAs) by using LC–MS/MS method. No PAs were detected in any of the batches (LOD ranging between 1 and 5 μg/kg) and thus below the maximum level for food supplements containing herbal ingredients (400 μg/kg).[4]

Regarding phytochemical analyses of the NF, the applicant provided CoAs with the following information: percentages of total flavonoids among six batches ranged between 18.34–28.32% (w/w) (based on the method by Miliauskas et al., 2004), total saponins (as ardisiacrispin A, which is highlighted by the applicant as the major triterpene saponin from *L. pumila*) among seven batches ranged between 0.49 and 0.70% (w/w) (measured by an LC–MS/MS-based method) and the total phenolic content among four batches ranged between 79.3 and 138.7 mg GAE/g (based on the method by Singleton and Rossi, 1965).

Since the NF is particulate in nature and is not soluble in water in all proportions, the applicant was requested to follow the guided pathway in the 'EFSA Guidance on technical requirements for regulated food and feed product applications to establish the presence of small particles including nanoparticles' (EFSA Scientific Committee, 2021) and produce the needed experimental evidence. Thus, the applicant conducted the water solubility test by following the OECD TG 105 (OECD, 1995) and in accordance with the GLP requirements. Based on the results of a preliminary test, a definitive test was carried out by using the shake flask method and the solubility of the NF was determined to be 216.01 g/L at 20°C. As per the above-mentioned EFSA Guidance (EFSA Scientific Committee, 2021), no additional assessment for the fraction of small particles was needed.

The Panel considers that the information provided on the composition of the NF is sufficient for characterising the NF.

[3] Analytical method for PAHs: gas chromatography–tandem mass spectrometry (GC–MS/MS) with the LOD/LOQ of 0.5 μg/kg; analytical method for pesticides: GC–MS/MS and liquid chromatography–tandem mass spectrometry (LC–MS/MS) with the LOQ of up to 0.5 mg/kg.
[4] Commission Regulation (EU) 2020/2040 of 11 December 2020 amending Regulation (EC) No 1881/2006 as regards maximum levels of pyrrolizidine alkaloids in certain foodstuffs. OJ L 420 14.12.2020, p 1.

3.4.1. Stability

In order to demonstrate the stability of the NF, the applicant conducted two studies to determine the shelf-life. The first study was conducted using four batches of the NF (#2, #3, #4 and #6) under accelerated conditions at 40°C ± 2°C and at 70 ± 5% RH (relative humidity) for a period of 6 months. The second study, using three batches of the NF (#1, #4 and #6), was conducted under normal storage conditions at 25°C and 60% RH, for a period of 24 months. The batches were analysed for appearance, moisture content (only in the first study under accelerated conditions), gallic acid content (only in the second study under normal conditions) and microbial stability.

The results of both studies indicate that the NF conforms to the selected physical, chemical and microbial specifications for at least its recommended shelf-life of 24 months and under storage conditions (in sealed containers, protected from light and humidity at temperature < 30 ± 2°C and RH < 65 ± 5%).

The Panel considers that the data provided sufficient information with respect to the stability of the NF during 24 months.

3.5. Specifications

The specifications of the NF are indicated in Table 2.

Table 2: Specifications of the NF

Description: Hydroalcoholic extract from a dried whole plant of *Labisia pumila* (Blume) Fern.-Vill. mixed with maltodextrin (as a drying aid) in a ratio 2:1	
Parameter	**Specifications**
Particle size	90% through 120 mesh (125 μm)
Ash	< 10%
Acid-insoluble ash	< 1%
Moisture	< 8%
Ethanol	< 1% (w/w)
Gallic acid	2–10% (w/w)
Carbohydrate	70–90 g/100 g
Protein	< 9% (w/w)
Total fat	< 3% (w/w)
Saponin (as ardisiacripsin A)	< 1.5% (w/w)
Aerobic plate count	$< 1 \times 10^4$ CFU/g
Yeast and mould	$< 5 \times 10^2$ CFU/g
E. coli	Not detected in 10 g
S. aureus	Not detected in 10 g
Salmonella	Not detected in 25 g
P. aeruginosa	Not detected in 10 g

CFU: colony forming units; w/w: weight per weight.

The Panel considers that the information provided on the specifications of the NF is sufficient and does not raise safety concerns.

3.6. History of use of the NF and/or of its source

3.6.1. History of use of the source

The plant *L. pumila* has a long history of traditional use in humans in Asia, especially in Malaysia, where it has been claimed to have beneficial putative effects in the treatment of numerous health conditions (Malaysian Herbal Monograph, 2022). The plant is traditionally boiled and the water extract is taken as a drink (FDPM, online).

3.6.2. History of use of the NF

One product containing the NF or the NF itself is sold in Malaysia, Singapore, Indonesia and the USA, as stated by the applicant.

Upon a request to address the safe consumption of the NF-containing products, which are available on the markets in certain countries, the applicant provided a letter from the Malaysian Ministry of Health related to post-market surveillance data. For one product containing the NF, self-reported data did not contain any report of adverse reactions or quality complaints.

3.7. Proposed uses and use levels

3.7.1. Target population

The target population proposed by the applicant is the general adult population (>18 years of age), excluding pregnant or lactating women.

3.7.2. Proposed uses and use levels

The applicant intends to market the NF for use in food supplements, at a maximum dose of 750 mg/day.

3.8. Absorption, distribution, metabolism and excretion (ADME)

The applicant investigated the bioavailability and pharmacokinetics of gallic acid (highlighted as key component of the NF) in Wistar rats (n = 4/sex per group) following oral gavage with 500 mg/kg body weight (bw) of the NF (equivalent to 1.43 mg/kg bw gallic acid) (Unpublished report, 2018a). Mean peak plasma concentrations were 63.86 ng/mL and 131.47 ng/mL in males and females, respectively, at a similar T_{max} of 0.083 h. The area under the curve (AUC_{0-t}) was 67.46 ng.h/mL in males and 155.99 ng.h/mL in females. The mean plasma terminal half-life was 1.14 and 1.16 h in males and females, respectively. The absolute oral bioavailability of gallic acid was estimated at 7% in males and 12% in females.

Additionally, as part of the 90-day toxicity study carried out with the NF (Unpublished study report, 2018b), Wistar rats received the NF via oral gavage in amounts of 0, 500, 1,500 and 2,000 mg/kg bw per day. While no gallic acid was found in pre-dose samples and in the control group on day 1 and 90, repeated oral administration of the NF demonstrated a dose-dependent increase in systemic exposure to gallic acid from day 1 to day 90 (C_{max} and AUC_{0-t}) in both sexes. Day 90/day 1 ratios for C_{max} and AUC_{0-t} suggest mild accumulation in females in the mid- and high-dose groups.

Finally, as part of the submitted 12-month chronic toxicity study with the NF (Unpublished study report, 2021c), Wistar rats received the NF via oral gavage in amounts of 0, 500, 1,000 and 1,500 mg/kg bw per day. Plasma was collected on days 1, 90, 180 and 365 of treatment. Overall, a time and dose-dependent increase in C_{max} and systemic exposure (AUC_{last}) was observed in males and females following administration of the test item, confirming the accumulation of gallic acid in both sexes.

3.9. Nutritional information

The applicant provided a nutritional analysis of the NF as indicated under the 'Compositional' section. According to the batch-to-batch analysis (Table 1), the NF mainly consists of carbohydrates (77.8–85.5%), ash (6.3–6.8%), protein (4.3–6.5%), moisture (3.5–5.1%) and fat (0.3–1.6%).

The Panel considers that taking into account the proposed conditions of use (i.e. max. 750 mg/day), consumption of the NF is not nutritionally disadvantageous.

3.10. Toxicological information

The applicant provided comprehensive toxicological information with several study reports on the NF, overviewed in Table 3. Some of the studies on genotoxicity were performed upon request from the Panel (see the following section).

Table 3: List of toxicological studies with the NF provided by the applicant

Reference	Type of study	Tested system	Doses
Genotoxicity			
Unpublished study, 2018c	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium TA98, TA100, TA102, TA1535 and TA1537	0, 312.5, 625, 1,250, 2,500 and 5,000 μg/plate (absence and presence of S9 mix)
Unpublished study, 2018d	*In vitro* chromosome aberration test (GLP, OECD TG 473)	Human lymphocytes	4 h preparation interval: 0, 125, 250 and 500 μg/mL (absence and presence of S9 mix); 22 h preparation interval: 0, 125, 250 and 500 μg/mL (absence of S9 mix)
Unpublished study, 2018e	*In vivo* mammalian erythrocyte micronucleus test (GLP, OECD TG 474)	Mice (polychromatic erythrocytes in the bone marrow)	0, 500, 1,000 and 2,000 mg/kg bw/day
Unpublished study, 2021a	*In vitro* mammalian cell micronucleus test (OECD TG 487)	Binucleated cultured human peripheral blood lymphocytes	0, 15, 30 and 60 μg/mL for 4 h (presence of S9-mix); 0, 41.75, 83.5 and 167 μg/mL for 4 h (absence of S9-mix); 0, 15, 30 and 60 μg/mL for 22 h (absence of S9-mix)
Unpublished study, 2021b	*In vitro* mammalian cell micronucleus test (GLP, OECD TG 487)	Binucleated cultured human peripheral blood lymphocytes	0, 75, 100, 125 and 150 μg/mL for 4 h (presence of S9-mix); 0, 100, 150, 200 and 250 μg/mL for 4 h (absence of S9-mix); 0, 75, 100, 150 and 225 μg/mL for 22 h (absence of S9-mix)
Subchronic and chronic toxicity			
Unpublished study, 2018b	90-day repeated dose oral toxicity study (GLP, OECD TG 408)	Wistar rats	0, 500, 1,500 and 2,000 mg/kg bw/day
Unpublished study, 2021c	Chronic oral toxicity study (GLP, OECD TG 452)	Wistar rats	0, 500, 1,000 and 1,500 mg/kg bw/day

bw: body weight; GLP: Good Laboratory Practice; OECD TG: Organisation for Economic Co-operation and Development Test Guideline.

In addition to the studies conducted with the NF, the applicant also carried out a literature search on toxicological data of different extracts of *L. pumila*, which was complemented by EFSA. Studies considered relevant to the safety assessment of the NF are described in the respective sections below.

The Panel notes that several *in vitro* studies available in the literature investigated potential oestrogenic properties of a range of *L. pumila* extracts that are different from the NF (Jamal et al., 2012; Poh et al., 2013; Muhamad et al., 2019). Evidence suggests that such extracts display estrogenic activity and contain compounds with binding affinities for oestrogen receptors Erα and Erβ.

3.10.1. Genotoxicity

A bacterial reverse mutation test (Unpublished study, 2018c) was performed using *Salmonella* Typhimurium tester strains TA98, TA100, TA102, TA1535 and TA1537 and the plate incorporation method (OECD TG 471). Tested strains were exposed to the NF at concentrations of 0 (vehicle control: dimethyl sulfoxide, DMSO), 312.5, 625, 1,250, 2,500 and 5,000 μg/plate, both in the absence and presence of a metabolic activation system (S9 mix). No increases in the numbers of revertant colonies were observed at any dose level compared to the negative (vehicle) control.

An *in vitro* mammalian chromosome aberration test using cultured human lymphocytes (Unpublished study, 2018d) was conducted with the NF (OECD TG 473). In the main experiment, cells were treated with the NF for 4 h (short exposure) with and without the S9 mix and for 22 h (continuous exposure) without the S9 mix at concentrations of 125, 250 and 500 μg/mL, and prepared for evaluation 22 h after the start of treatment. Those doses were selected based on solubility and precipitation of the NF and the results of a preliminary cytotoxicity assay (using the % Mitotic Index, MI). Regarding the results of the main test, the short-term exposure resulted in a dose-dependent

reduction in % MI, ranging between 18–25% and 42–50% (with and without S9 mix) compared to the negative control (vehicle: DMSO) in the absence and presence of the S9 mix, respectively. After continuous exposure, the MI was reduced to 41–48% of that in the negative control cultures. None of the three treatment regimens and concentrations exhibited a statistically significant increase in the frequency of structurally aberrated cells compared to the negative control. Positive controls (mitomycin C and cyclophosphamide) produced the expected responses.

An *in vivo* mammalian erythrocyte micronucleus test in mice following oral gavage administration of the NF (OECD TG 474) (Unpublished study, 2018e) was submitted by the applicant. Mice (n = 5/sex per group) received cyclophosphamide (positive control, once), purified water (negative control), and the NF for 2 consecutive days at the dose levels of 500, 1,000 and 2,000 mg/kg bw. No mortality or abnormal clinical signs were observed in any of the dose groups and negative control. Bone marrow cells were collected approx. 24 h after final dose administration and evaluated for the occurrence of micronuclei. The ratio between polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) was determined to evaluate cytotoxicity and reported as the number of PCEs per 500 erythrocytes. The NF did not exert any cytotoxic effects in the bone marrow. No statistically significant increase in percent micronucleated (MN) cells was observed in any of the dose groups as compared to the negative control. The positive control produced the expected response. The Panel considers that, based on the unchanged ratio between PCEs and NCEs and the absence of bone marrow toxicity, it is unknown whether and to what extent the substance(s) of the NF have reached the target tissue and thus, this study is not sufficient to properly address the genotoxic potential of this NF.

Following an EFSA request, and in accordance with the EFSA recommendations for genotoxicity testing (EFSA Scientific Committee, 2011), the applicant submitted an *in vitro* mammalian cell micronucleus test following OECD TG 487 and in compliance with GLP principles (Unpublished study, 2021a). Based on a preliminary cytotoxicity assay (cytokinesis-block proliferation index – CBPI, reduction and at least 50% cytotoxicity in terms of inhibition of cell proliferation), binucleated cultured human peripheral blood lymphocytes were exposed to the NF under the following conditions: short-term (approx. 4 h) exposure to 15, 30 and 60 μg/mL (with the S9 mix) and to 41.8, 83.5 and 167 μg/mL (without S9 mix) and continuous exposure (approx. 22 h) to 15, 30 and 60 μg/mL (without S9 mix). Precipitation was not observed in any of the treatment regimens. No increase in mean percent MN binucleated cells in the exposed cells compared to the control (DMSO) was observed under any of the treatment regimens. The positive control substances induced statistically significant increases in the mean percentage of MN binucleated cells compared to the negative control. The Panel notes that, according to OECD TG 487, if the cytokinesis blocker cytochalasin B (cytoB) is used (as was the case in the present study), the maximum concentration of the test substance should aim at reduction of the CBPI to 45% (±5%) of the concurrent negative control. Thus, a comparable level of cytotoxicity was not achieved with the highest concentrations tested in the main test (only 12% cytotoxicity at 60 μg/mL with S9-mix, only 33% at 167 μg/mL without S9-mix in short-term exposure and only 38% at 60 μg/mL without S9-mix in continuous exposure). In addition, since the NF can be regarded as a mixture containing a number of different constituents, the Panel considers that sufficiently high concentrations must be applied (in compliance with the OECD TG 487). The Panel also considers that when testing higher concentrations, adjustment of the dose spacing (a closer spacing) should be applied to account for the possibility of a steep dose–response.

Upon a request, the applicant conducted a new *in vitro* mammalian cell micronucleus test following OECD TG 487 and in compliance with GLP principles. Based on a previous study (Unpublished study, 2021b), binucleated cultured human peripheral blood lymphocytes were exposed to the NF under the following conditions: short-term (approx. 4 h) exposure to 75, 100, 125 and 150 μg/mL (with S9 mix) and to 100, 150, 200 and 250 μg/mL (without S9 mix) and continuous exposure (approx. 22 h) to 75, 100, 150 and 225 μg/mL (without S9 mix). Precipitation was not observed in any of the treatment regimens. Cytotoxicity was observed at the highest concentrations of all regimens (67% at 150 μg/mL with S9-mix, 58% at 250 μg/mL without S9-mix in short-term exposure and 64% at 225 μg/mL in continuous exposure), thus those concentrations were omitted from counting the number of MN binucleated cells. No dose-dependent or significant increase in mean percent MN binucleated cells in the exposed cells compared to the control (DMSO) was observed under any of the remaining treatment regimens. The positive control substances induced statistically significant increases in the mean MN binucleated cells compared to the negative control.

Overall, the Panel considers that the studies provided do not raise concerns regarding the genotoxicity of the NF.

3.10.2. Acute and subacute toxicity

Both the acute and subacute toxicity studies were conducted using a similar *L. pumila* extract produced with minor deviations from the production process described in Section 3.3, but with the same extraction method. Those are not expected to affect the composition of the NF, and therefore the results of the studies.

The applicant carried out an acute oral toxicity study (Saeed et al., 2018) according to OECD TG 425 (2008). Briefly, a *L. pumila* standardised extract was orally administered to Sprague–Dawley rats (n = 5/group) in single doses of 0 or 2,000 mg/kg bw. Animals were monitored for 14 days. No lethal or behavioural effects were observed, and no statistically significant changes were noted in organ weights relative to body weights.

The applicant also carried out a subacute oral repeated dose toxicity study (Saeed et al., 2018) according to OECD TG 407 (2008). Briefly, Sprague–Dawley rats (n = 5/sex per group) received 0, 250, 500 or 1,000 mg/kg bw per day of a *L. pumila* standardised extract for 28 days, by oral gavage. No lethal, clinical or functional effects were observed. Weekly body weight was significantly decreased in females exposed to 500 (−7%) mg/kg bw per day of the test compound on day 14. Organ-to-body weight ratios at necropsy remained unaffected. Haematological and biochemical parameters remained unaffected as well, except for an isolated increase in triglyceride levels (+28%) in the low dose group. No renal or hepatic histopathological effects were observed.

Considering these data, the applicant expected a high dose of 2,000 mg/kg bw per day to show minimal toxicity and selected it as the highest dose for subchronic toxicity testing (cf. Section 3.10.3).

3.10.3. Subchronic toxicity

The applicant provided a 90-day subchronic oral toxicity study with the NF (Unpublished study, 2018b). The study was conducted in accordance with OECD TG 408 (OECD, 1998) and in compliance with the principles of GLP. Wistar rats (n = 10/sex per group) were administered the NF at doses of 0 (vehicle control: purified water), 500 (low-dose), 1,500 (mid-dose) and 2,000 (high-dose) mg/kg bw per day for 90 days by oral gavage. Five additional animals per sex, receiving 0 and 2,000 mg NF/kg bw per day, were kept for a 28-day recovery period. The Panel notes the narrow dose spacing between the middle- and high-dose groups.

Neither lethality nor ophthalmological, neurobehavioural or functional effects were observed.

Weekly mean body weight was statistically significantly altered in males from the mid-dose group starting from week 4, resulting in a terminal body weight reduced by 13.5% ($p < 0.01$). This was associated with a significant decrease in feed consumption within that same group, and occasionally in the high-dose group. Feed efficiency calculations as provided by the applicant were inconclusive.

The Panel notes that statistically significant differences in haematology parameters between controls and different dose-groups (across the sexes) were observed. However, they were not seen in all dose groups and were not dose-dependent. In addition, they were small in magnitude (within historical control ranges) and reversible (based on the data from recovery groups). Thus, these findings could be considered incidental.

A number of statistically significant differences in serum clinical chemistry parameters across the sexes between controls and animals exposed to the NF were observed. These parameters included: total protein, albumin, globulin, albumin/globulin ratio, sodium, calcium, phosphorus, glucose, total bilirubin and creatinine. A dose-dependent decrease in total protein (up to −9%) was observed in males, and the effect was reversible after recovery. Decreases in globulin and calcium were also present after recovery. However, none of the observed differences were considered biologically relevant since they were not dose-dependent and/or small in magnitude (within historical control ranges).

Statistically significant differences were observed in organ weights in males, including:

- A decrease in absolute liver weight in the mid- and high-dose groups (−15 and −14%, respectively), corroborated by a decrease in liver weight relative to body weight in the high-dose group (−9%)
- A decrease in absolute prostate weight in the mid- (−25%) and high-dose (−14%) groups
- An increase in relative testicular weight in the mid- (+14%) and high dose (+12%) groups
- An increase in relative thyroid and parathyroid weights in the mid- (+32%) and high-dose groups (+22%)

- An increase in relative heart (+12%), brain (+13%) and adrenal (+17%) weights in the mid-dose group only

Analyses of thyroid hormone levels at necropsy revealed an isolated statistically significant decrease in thyroid-stimulating hormone (TSH) levels in the low-dose group of females (−34%). In males, thyroxine (T4) levels displayed a dose-dependent decrease in all groups, reaching statistical significance at the high-dose (−24%). An increase in TSH levels in the mid- and high-dose group males, although not statistically significant, suggests a compensatory reaction to the inhibition of T4 production. Following recovery, TSH levels in the high-dose group males were significantly lower than the control (−56%), while T4 levels were unaffected. These effects are associated with a statistically significant increase in the relative weight of the thyroid and parathyroid glands in the mid- and high-dose groups in males, but no related histopathological changes were observed. Considering the inter-individual variability and unclarity of trends observed in thyroid hormone levels, the Panel considers that the results regarding thyroid effects are inconclusive.

No statistically significant difference in the number of oestrous cycles was observed during the last 2 weeks prior to necropsy. However, oestrous cyclicity length was slightly increased in all treated females (up to +11.5%; not statistically significant). Individual data show a slight trend in prolongation of the dioestrus phase of the cycle (2 days instead of 1 day) with the incidence of 0/10 animals in the control, 3/10 in the low-dose, 4/10 in the mid-dose and 3/10 animals in the high-dose group.

In light of literature data showing potential endocrine activity of various extracts from *L. pumila* (Jamal et al., 2012; Poh et al., 2013; Muhamad et al., 2019), and considering the observed effects on oestrous cyclicity and changes in testicular and prostatic weights, the Panel had a concern that the NF may contain substances with endocrine activity. Thus, the applicant was asked to conduct further analyses in serum samples collected from the rats for several hormone levels that were not investigated originally. Since as per the study plan, serum samples were discarded upon finalisation of the study, further analyses were not possible. However, the applicant later provided a chronic oral toxicity study (OECD TG 452; cf. Section 3.10.4).

3.10.4. Chronic toxicity

As a part of the authorisation process of the NF in the US market, the applicant conducted a chronic oral toxicity study (OECD TG 452; Unpublished study, 2021c), which was made available to EFSA. The study was conducted in compliance with the principles of GLP (OECD, 1998a). Wistar rats (n = 20/sex per group) were administered the NF at doses of 0 (vehicle control: purified water), 500, 1,000 and 1,500 mg/kg bw per day by oral gavage for 1 year. In addition, interim groups (n = 10/sex per dose) went through the same regimen and were sacrificed after 6 months. Recovery groups (n = 10/sex per group), comprised of the control and high-dose groups (1,500 mg/kg bw per day), were also included to assess reversal or delayed test item-related changes after a 1-month recovery period.[5]

Terminal body weights of all treated males (interim, main and recovery groups) were lower in comparison to their respective controls (reaching a statistically significant difference only in the high-dose group after 6 months; −14%).

As feed consumption was also reduced, the applicant was requested to calculate feed efficiency for a better interpretation of reduced feed consumption and body weight gains. The Panel notes that the provided feed efficiency data did not allow to differentiate treatment-related from feed consumption-related effects since it was calculated on a weekly basis and was inconclusive.

Observations related to haematological parameters revealed a statistically significant decrease in absolute lymphocytes in the high-dose groups in females at the end of the third month of treatment. After 6 months of treatment, a dose-dependent decrease in reticulocytes count (reaching statistical significance in the mid- and high-dose groups) in males was observed. Finally, several haematological (platelet count, MCHC, total lymphocytes, total red blood cells and total eosinophils) and clinical chemistry (total cholesterol, total bilirubin, creatinine, sodium) parameters were statistically significantly changed in treated animals. None of the observed differences were considered to be of toxicological concern since they were not dose-dependent and/or small in magnitude.

Statistically significant differences were observed in organ weights in treated males, including dose-dependent decreases in the absolute liver, kidney, and brain weights, which were generally not

[5] As mentioned and described under the Section 3.8 ADME, as a part of this chronic toxicity study, toxicokinetic parameters were also evaluated in additional groups of rats.

maintained when expressed relative to body weight. A dose-dependent increase in relative testicular weight was observed in all groups, reaching statistical significance in the main (+14.5%) and recovery (+21.6%) high-dose groups. A similar pattern was observed for the relative weight of epididymides. Findings were within the historical range.

These findings were accompanied by a dose-dependent increasing incidence of diffuse degeneration/atrophy in testes (1/20, 2/20, 4/20 and 5/20 cases in ascending dose groups, respectively), and an increased incidence in reduced sperm (1/20, 1/20, 3/20, 3/20 cases in ascending dose groups, respectively) and cell debris (1/20, 1/20, 2/20, 2/20 cases in ascending dose groups, respectively) in epididymides. Recovery animals, however, displayed higher incidences in the control group than in the high-dose group for those three parameters. Further histopathological analyses suggest that such testicular effects could be incidental.

In females, absolute ovarian weight was significantly decreased in the low- and mid-dose groups after 6 months of treatment, but such effect was not maintained after 12 months or recovery, or when ovarian weight was expressed relative to body weight. Uterine weight, both absolute and relative, was significantly increased in the high-dose group after 12 months (up to +48%) and remained increased after recovery. Absolute thyroid and parathyroid gland weight was increased in a statistically significant manner in all treated females after 12 months, but such effect was not maintained when expressed relative to body weight. T4 levels were occasionally increased in the mid-dose group after 9 months of treatment, but no clear trends were notable in thyroid hormone levels. No hormone levels other than thyroid hormones and TSH could be provided by the applicant.

The Panel notes that similar effects were observed in the subchronic and chronic toxicity studies. Dose-dependent decreases in male body weights were associated with decreases in food consumption, but feed efficiency calculations were inconclusive. Additionally, treated males displayed a dose-dependent increase in relative testicular weights, but it remains unclear to which extent this effect is adverse or associated with the observed decrease in body weight. The Panel notes that several other endpoints linked to hormonal function – including oestrous cyclicity, thyroidal, uterine, prostatic and epididymal weights, or thyroid hormone levels – were occasionally affected throughout both studies, with statistical significance starting at 1,000 mg/kg bw per day. While dose–response relationships were not clearly established for such endpoints, the Panel considers that the NF may display endocrine activity, as previously described in the literature (Jamal et al., 2012; Poh et al., 2013; Muhamad et al., 2019). The Panel identifies an overall NOAEL of 500 mg/kg bw per day, the lowest dose tested, for the NF.

3.10.5. Reproductive and developmental toxicity

3.10.5.1. Reproductive toxicity

Oestrous cyclicity and reproductive parameters were investigated in nulliparous Sprague–Dawley rats administered 0 (vehicle; distilled water), 20, 200 or 1,000 mg/kg bw per day of an aqueous extract of *L. pumila* for a duration of 3 weeks (Mohd Fuad et al., 2017). The treatment did not cause mortality nor cause noticeable toxicity on the physical appearance, behaviour and body weight of treated females. The mean length of the oestrous cycle displayed a dose-dependent, non-statistically significant trend towards an increase, and a higher incidence of irregular cycles was observed in the high-dose group (1/10, 1/10, 0/10 and 3/10 in ascending dose groups, respectively). No statistically significant difference or clear trend was observed in hormonal levels (17β-oestradiol, progesterone and free testosterone) at necropsy. No indications of abnormalities in the histology of uterus and vagina were observed. However, the presence of ovarian follicular cysts was observed in 4/10 animals in the high-dose group compared to 1/10 in the control group.

The Panel notes that the observations made on oestrous cyclicity are comparable to those made in the subchronic toxicity study conducted with the NF.

3.10.5.2. Developmental toxicity

Pregnant Sprague–Dawley dams were treated with 0 (vehicle; distilled water), 2, 20, 200, 400 or 1,000 mg/kg bw per day of a *L. pumila* standardised aqueous extract from gestational day (GD) 6 to GD16, and were euthanised at GD21 (Mohd Fuad et al., 2005). Of all monitored endpoints (i.e. gravid uterine weight, number of corpora lutea, number of implantation sites, percentage of fetal resorptions, number of live fetuses, fetal weight, fetal sex ratio and congenital malformations), no statistically significant effect or clear trend was observed.

Females Sprague–Dawley rats were treated with an aqueous extract of *Labisia pumila* at 0 (vehicle; distilled water), 2, 20, 200, 400 or 800 mg/kg bw per day by gavage 10 days prior to mating and during mating (max. 10 days), gestation and lactation (Wan Ezumi et al., 2007). Both the dams and the pups were euthanised on the post-natal day (PND) 7. The treatment did not affect general health or the oestrous cycle of females, which all proceeded towards successful pregnancy. No changes in maternal and fetal body weights, number of implantations, litter size, sex ratio, live birth index or pup viability index were observed. Mean pregnancy duration was lower in animals treated at doses of 20 mg/kg bw per day and above, but no dose-dependency or statistical significance was observed.

The Panel notes that these studies do not raise concerns regarding the embryotoxicity and teratogenicity of *L. pumila* standardised aqueous extracts.

3.10.6. Human data

The applicant provided a case-report study conducted with the product containing the NF (Abdul Majid et al., 2019). One obese female (BMI = 33.2 kg/m^2; 59 years of age) with medication-controlled hypothyroidism was receiving the NF (250 mg/day, i.e. 125 mg twice daily after meals for 40 days). The only reported treatment-related adverse effect was a mild sensation of bloating and gas (during the first week of study). Except for a slight fluctuation in uric acid (from 393 to 410 μmol/L; normal range is 150–357 μmol/L), no other changes in haematological and biochemical parameters were noted after the last dosage in comparison to the baseline values.

The applicant also provided a safety report of a randomised, double,-blind, placebo-controlled, dose-range finding study (Unpublished study report, 2022). In this Phase-2 study, the NF was given to subjects (160 subjects recruited, 120 completed the study) with BMI > 25 kg/m^2, otherwise healthy, who were divided in 4 groups receiving either placebo or the NF, twice daily in amounts of 750 mg/day, 1,125 mg/day and 1,500 mg/day for the period of 12 weeks. The primary and secondary endpoint was to investigate the efficacy of the NF in improving different parameters regarding obesity, while safety endpoints included the incidence of abnormal vital signs (heart rate and blood pressure), laboratory test results (haematology, fasting blood glucose, HbA_{1C}, electrolytes, lipid profile, liver and renal function tests and urinalysis) and adverse events. The number of adverse events was not higher in any of the dose groups in comparison to placebo and most of them were categorised by the investigators as mild and moderate, as well as unlikely and possibly related to the NF. No clinically significant changes in laboratory parameters were observed.

Except for the above-mentioned studies, the applicant provided a literature review of the human studies conducted with aqueous extracts of the *L. pumila* plant (as a whole or parts of it). Those studies are summarised in Appendix A.

The Panel notes that the human studies provided by the applicants and those found by an extensive literature search were primarily designed to investigate putative beneficial effects at intakes up to 1,500 mg/day and addressed only a limited number of safety-relevant endpoints. Several studies available from the literature, which included an investigation on hormonal levels (Abdul Kadir et al., 2012; George et al., 2014; Norhayati et al., 2014), were administering doses of aqueous extracts of *L. pumila* at levels (up to 400 mg/day), which were below the proposed daily intake of NF in the current application (up to 750 mg/day), although direct dose-comparison with the NF is not possible. The Panel notes that no changes were found in the studied safety-related parameters and no adverse events related to the consumption of the NF were reported. The Panel notes, however, the inherent limitations of such human studies for their use in this safety assessment.

3.11. Allergenicity

The applicant referred to the human data submitted in the previous section and the absence of reported allergic reactions. In addition, the applicant also reported one article (Jamia Azdina, 2000; only an abstract available), which investigated contact dermatitis caused by *L. pumila* plant using a TROLAB patch test. Positive controls as well as the leaf and root of the plant were patched onto the skin of human subjects. After 48 h, slight blisters were observed on patches containing the root. The condition aggravated after 96 h and slight blisters were also observed with the leaf.

Upon a request, the applicant performed a literature review of the botanical relatedness of the source of the NF (*L. pumila*) with other plant species with regard to cross-reactivity. The review was focused on the plant species of the Myrsinaceae family. In short, a few case-reports (Bolhaar et al., 2000; Ariano et al., 2006) were retrieved on an allergy to the pollen of *Cyclamen* genus, developed

due to occupational exposure and one case-report (Lin et al., 2019) of contact dermatitis, due to topical use of *Lysimachia clethroides*. This information does not predict allergenicity of the NF.

The Panel considers that there is insufficient basis to conclude on the risk of allergenicity for the NF, but given the protein content, some risk may be present.

4. Discussion

The NF, which is the subject of this application, is an aqueous ethanolic extract (1:1) of the whole plant of *L. pumila*, mixed with maltodextrin (used as a drying aid). The applicant intends to market the NF as a food supplement in a dose up to 750 mg/day. The target population proposed by the applicant is the general adult population (except pregnant and lactating women).

Taking into account the composition of the NF and the proposed conditions of use, consumption of the NF is not nutritionally disadvantageous.

The Panel considers that the NOAEL, which was identified based in the subchronic and chronic studies with the NF, is 500 mg/kg bw per day. By applying an uncertainty factor of 100 [10 (interspecies variability) × 10 (intraspecies variability)], the Panel considers an intake up to 5 mg/kg bw per day as safe.

For the target population (adults excluding pregnant and lactating women) with a default body weight of 70 kg (EFSA Scientific Committee, 2012), the intake of 5 mg/kg bw per day corresponds to 350 mg of the NF per day. This intake is lower than the use level proposed by the applicant.

5. Conclusions

The Panel concludes that the NF, aqueous ethanolic extract (1:1) of the whole plant of *L*. pumila mixed with maltodextrin (2:1) which serves as a drying aid, is safe for the target population at levels up to 350 mg/day.

5.1. Request for protection of proprietary data in accordance with Article 26 of Regulation (EU) 2015/2283

The Panel could not have reached the conclusion on the safety of the NF under the proposed conditions of use without the following data claimed as proprietary by the applicant: the solubility test of the NF and toxicological information (studies on pharmacokinetics, genotoxicity, subchronic and chronic oral toxicity).

6. Steps taken by EFSA

1) On 14/04/2020 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of an aqueous extract of *Labisia pumila* as a novel food. Ref. Ares(2020)2044353–14/04/2020.
2) On 14/04/2020, a valid application on aqueous ethanolic standardised extract of *Labisia pumila*, which was submitted by Orchid Life Sdn Bhd, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1337) and the scientific evaluation procedure was initiated.
3) On 26/02/2021, the applicant informed EFSA about the change in the name of the company to Medika Natura Sdn. Bhd.
4) On 27/08/2020, 26/02/2021, 18/03/2021, 23/04/2021, 24/01/2022, 5 June 2022, 24/05/2022 and 21/07/2022, EFSA requested the applicant to provide additional information or to clarify previously submitted one, in order to accompany the application and the scientific evaluation was suspended.
5) On 26/02/2021, 4 June 2021, 22/12/2021, 5 June 2022, 13/06/2022 and 25/08/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
6) During its meeting on 28/09/2022, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of an aqueous ethanolic extract of *Labisia pumila* as a NF pursuant to Regulation (EU) 2015/2283.

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Abbreviations

AACC	American Association of Cereal Chemists
AAS	atomic absorption spectrophotometry
ADME	absorption, distribution, metabolism and excretion
AOAC	Association of Official Agricultural Chemists
AUC	area under the curve
BLAST	The Basic Local Alignment Search Tool
BMI	body mass index
bw	body weight
CBPI	cytokinesis-block proliferation index
CFU	colony forming units
CoA	certificate of analysis
CytoB	cytochalasin B
DMSO	dimethyl sulfoxide
FD	fluorescence detector
FRIM	Forest Research Institute Malaysia
GAE	gallic acid equivalents
GC–MS/MS	gas chromatography–tandem mass spectrometry
GD	gestational day
GLP	Good laboratory practice
GMP	Good Manufacturing Practice

HACCP	Hazard Analysis Critical Control Points
HPLC-UV/ViS	high-performance liquid chromatography with ultraviolet/visible light detector
ISO	The International Organization for Standardization
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LOD	limit of detection
LOQ	limit of quantification
MCHC	mean corpuscular haemoglobin concentration
MI	mitotic index
MN	Micronucleated
MyGAP	Malaysia Good Agricultural Practices
NCEs	normochromatic erythrocytes
NDA	EFSA Panel on Nutrition, Novel Foods and Food Allergens
NF	novel food
NOAEL	no observed adverse effect level
OECD TG	Organisation for Economic Co-operation and Development Test Guideline.
PAHs	polycyclic aromatic hydrocarbons
PAs	pyrrolizidine alkaloids
PCB	polychlorinated biphenyl
PCE	polychromatic erythrocyte
PND	post-natal day
RH	relative humidity
SD	standard deviation
T4	thyroxine
TSH	thyroid-stimulating hormone
US FDA	The United States Food and Drug Administration
WHO-PCDD/F-PCB-TEQ	sum of polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans-polychlorinated biphenyls expressed as World Health Organization toxic equivalent
w/w	weight per weight

Appendix A – Overview of human studies

Reference	Study design	Study population	Duration of Study	Doses	Safety-related parameters investigated
Hussain et al., 2009	Randomised, double-blind, placebo-controlled	Post-menopausal healthy Malay women (n = 70); power calculations performed based on the increase in mean testosterone levels and changes in BMI and blood pressure	6 months	0, 140, 280 and 560 mg/day of water extract of *L. pumila* (BioLabisia™)	BP, pulse rate, BMI, WHR, cardiovascular, breast, abdomen and pelvic examination and pap smear, ECG and chest x-ray, haematology (full blood count, APTT and PT), biochemistry (urea, creatinine, ALT and AST), adverse events
Abdul Kadir et al., 2012	Randomised, double-blind, placebo-controlled	Post-menopausal Malay women (n = 63)	6 months	0 and 280 mg/day of water extract of *L. pumila* (1:6)	Menopausal symptoms, BP, BMI, WC, fasting blood sugar, lipid profile, hormonal profile (FSH, LH and oestradiol)
Norhayati et al., 2014	Randomised, double-blind, placebo-controlled, parallel group	Healthy pre- and post-menopausal women (n = 197). Power calculation based on the oestradiol level	16 weeks	0 and 400 mg/day of water extract of *L. pumila* (BIOLP101)	Hormonal profile (IGF, FSH, LH and 17β-oestradiol), ALT, AST, cardiovascular risk factors, total bone mineral density, adverse events
George et al., 2014	Randomised, double-blind, placebo-controlled	Healthy pre- and post-menopausal North American women (n = 36)	12 weeks	0 and 200 mg/day of BIOLP101™	Lipid profiles, anti-inflammatory markers, urinary antioxidants, 17β-oestradiol, blood chemistry, AST, ALT, GGT, CBC, weight, vital signs, adverse events