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SCIENTIFIC OPINION

Safety of ashitaba sap as a Novel food pursuant to Regulation (EU) 2015/2283

EFSA Panel on Nutrition, Novel Foods and Food Allergens (NDA) | Dominique Turck | Torsten Bohn | Jacqueline Castenmiller | Stefaan De Henauw | Karen Ildico Hirsch-Ernst | Alexandre Maciuk | Inge Mangelsdorf | Harry J. McArdle | Androniki Naska | Kristina Pentieva | Alfonso Siani | Frank Thies | Sophia Tsabouri | Marco Vinceti | Margarita Aguilera-Gómez | Francesco Cubadda | Thomas Frenzel | Marina Heinonen | Miguel Prieto Maradona | Monika Neuhäuser-Berthold | Morten Poulsen | Josef Rudolf Schlatter | Alexandros Siskos | Henk van Loveren | Maria Glymenaki | Eirini Kouloura | Helle Katrine Knutsen

Correspondence: nif@efsa.europa.eu

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 ashitaba sap as a novel food (NF) pursuant to Regulation (EU) 2015/2283. Ashitaba sap is collected from harvested stems of *Angelica keiskei* plants. The principal constituents of the sap with regard to the safety assessment are chalcones (1%–2.25%) and furanocoumarins (< 0.01%). The applicant proposed to use the NF in food supplements at a maximum dose of 780 mg per day. The target population is adults excluding pregnant and lactating women. Taking into consideration the composition of the NF and the proposed uses, the composition of the NF is not nutritionally disadvantageous. There are no concerns regarding genotoxicity of the NF. Based on a 90-day oral toxicity study performed with the product as intended to be placed on the market (30% ashitaba sap powder and 70% cyclodextrins), the Panel establishes a safe dose of 0.5 mg/kg body weight (bw) per day for the product as it is intended to be placed on the market. For the target population, i.e. adults, this safe dose corresponds to 35 mg per day of the product as it is intended to be placed on the market and 137 mg per day of the NF, which is lower than the use level proposed by the applicant. The Panel concludes that the NF is safe for the target population at intake levels up to 137 mg per day.

KEYWORDS

Angelica keiskei, ashitaba, food supplement, novel foods, safety

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1 | INTRODUCTION

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

On 8 August 2019, the company Japan Bio Science Laboratory (JBSL)-USA, Inc. submitted a request to the Commission in accordance with Article 10 of Regulation (EU) No 2015/2283[1] to place on the EU market ashitaba (*Angelica keiskei* Koidzumi plant) sap powder as a novel food (NF).

The ashitaba (*Angelica keiskei* Koidzumi plant) sap powder final product (30% ashitaba sap powder and 70% cyclodextrins) is intended to be used in food supplements as defined in Directive 2002/46/EC[2] for adults, excluding food supplements for pregnant and lactating women.

In accordance with Article 10(3) of Regulation (EU) 2015/2283, on the 19th of December 2019, the European Commission asked the European Food Safety Authority to provide a scientific opinion on ashitaba (*Angelica keiskei* Koidzumi plant) sap powder as a NF.

1.2 | Interpretation of the Terms of Reference

In the course of the risk assessment, the Panel noted that the product which was the subject of this application was composed of 30% ashitaba sap and 70% cyclodextrins; therefore, the Panel considered the novel part of the product as it is intended to be placed on the market as the NF, i.e. the ashitaba sap part excluding the cyclodextrins. In consequence, the applicant was requested to modify the dossier accordingly and provide additional data.

2 | DATA AND METHODOLOGIES

2.1 | Data

The safety assessment of this NF is based on data supplied in the application and information submitted by the applicant following EFSA requests for supplementary information. During the assessment, the Panel identified additional data which were not included in the application.

Administrative and scientific requirements for NF applications referred to in Article 10 of Regulation (EU) 2015/2283 are listed in Commission Implementing Regulation (EU) 2017/2469.[3]

A common and structured format on the presentation of NF applications is described in the EFSA guidance on the preparation and presentation of an 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 characterisation of botanical source, certificates of raw materials, methods of analysis, certificates of analysis, two bacterial reverse mutation tests (Krul, 2002, unpublished; Joshi, 2023a, unpublished), an in vitro mammalian chromosomal aberration test (de Vogel, 2003, unpublished), an in vitro mammalian cell micronucleus test (Joshi, 2023b, unpublished), an in vivo micronucleus test (Cardoso & Licollari, 2014, unpublished), an acute oral toxicity study (Prinsen, 2002, unpublished), two 90-day oral toxicity studies (Oda, 2006, unpublished; Kukulinski, 2013, unpublished), a report on histopathological findings observed in a 90-day oral toxicity study (Seely, 2012, unpublished), a human study (Tomita, 2017, unpublished).

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 Commission Implementing Regulation (EU) 2017/2469.

This assessment concerns only the risks that might be associated with the 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.

[1]Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/ 2011 of the European Parliament and of the Council and repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001 (2013/0435 (COD). OJ L 327, 11.12.2015, p. 1–22.

[2]Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements. OJ L 183, 12.7.2002, p. 51–57.

[3]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.

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3 | ASSESSMENT

3.1 | Introduction

The NF according to the applicant, as defined by Regulation (EU) 2015/2283, Article 3, falls under the category 'Foods consisting of, isolated from or produced from plants or their parts'.

The NF which is the subject of the application is the sap from the *Angelica keiskei* plant. The NF consists primarily of water, with lower concentrations of fat, ash and protein. In addition, a range of 1%–2% of chalcones constituted by two compounds, namely xanthoangelol and 4-hydroxyderricin, are present in the NF. The NF is proposed to be used as an ingredient in food supplements at a maximum dose of 780 mg/day. The target population is adults, excluding pregnant and lactating women.

3.2 | Identity of the NF

The NF is the viscous yellow liquid sap of the *Angelica keiskei* Koidzumi plant. *A. keiskei* is commonly named ashitaba and belongs to the Apiaceae family. The ashitaba sap is collected from harvested stems of ashitaba plants cultivated in Indonesia. The identity of the plant used for the production of the NF is confirmed by comparison to authenticated samples of *A. keiskei* using macroscopic and microscopic methods based on consistent characteristic cellular structures of distinct striations radiating from the stomata of the leaf. Moreover, the phytochemical fingerprint of the plant material is compared to an authenticated reference sample of *A. keiskei* using high-performance thin layer chromatography (HPTLC).

3.3 | Production process

The NF is the sap of cultivated *A. keiskei* plants originating from certified organic farming. The sap is extracted from stems of mature ashitaba plants by hand squeezing, and subsequently undergoes pasteurisation to obtain the NF. In later stages, the NF is mixed with food grade cyclodextrin (composed of linear α-, β-, γ-cyclodextrin, branched cyclodextrins and oligosaccharides), sterilised, freeze-dried and then sieved to formulate the product as it is intended to be placed on the market, which is composed of around 30% ashitaba sap powder and 70% cyclodextrin, standardised for chalcone content (at least 8%) by adjusting the amount of cyclodextrin. As the freeze-drying of the liquid ashitaba sap results in the concentration of sap constituents, the chalcone content in the dried powder amounts to about 8%; thus, the percentage of chalcones in the product as it is intended to be placed on the market is higher than in the liquid sap. The production of the NF involves only physical processes and techniques and does not involve the use of solvents or processing aids; therefore, the production process is not considered novel.

The applicant mentioned that the NF is produced in line to good manufacturing practice (GMP) and provided the ISO 9001:2008 and ISO 20000:2005 certifications.

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

3.4 | Compositional data

The NF is the sap from *Angelica keiskei* plant, and it is composed on average of 92.9% water, 0.2% fat, 0.3% protein and 0.2% ash.

In order to confirm that the manufacturing process is reproducible and adequate to produce on a commercial scale a product with the required characteristics, the applicant provided proximate analyses and analytical data on the content of chalcones for five independently produced batches of the NF (Tables 1 and 2). In these batches, the percentage of chalcones, expressed as the sum of xanthoangelol and 4-hydroxyderricin, was on average 1.8%.

TABLE 1 Batch to batch proximate analysis of five batches of the NF.

	Batch number					
Parameter (%)	**#1**	**#2**	**#3**	**#4**	**#5**	**Method of analysis**
Water	93.1	93.7	93.3	91.6	92.7	Gravimetry (internal method)
Carbohydrates	6.3	5.5	6.1	7.7	6.8	Calculation (internal method)
Fat*	≤ 0.1	0.25	0.15	0.24	0.12	Gravimetry (internal method)
Protein	0.34	0.29	0.20	0.34	0.24	Titrimetry (Kjeldahl method, internal method)
Ash	0.3	0.2	0.2	0.2	0.2	Gravimetry (internal method)
Total	100.1	99.9	100.0	100.1	100.1	

*Chalcones are expected to be partially included in the fat measurements; gravimetric method used is unspecific.

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TABLE 2 Analyses of chalcones in five batches of the NF.

Parameter (%, w/v)	Batch number #1	#2	#3	#4	#5	Method of analysis
Xanthoangelol	1.3	0.67	1.1	1.3	1.3	HPLC (internal method)
4-Hydroxyderricin	0.72	0.38	0.64	0.74	0.76	HPLC (internal method)
Total Chalcone	2.02	1.05	1.74	2.02	2.06	Sum of xanthoangelol and 4-hydroxyderricin

Abbreviation: HPLC, high-performance liquid chromatography.

Plants from the *Angelica* spp. have the potential to contain coumarins and furanocoumarins in their fruit, roots and other underground parts and other live parts of the plant (EFSA, 2012). Compositional data for coumarin content were provided for five batches of the NF (Table 3), in which the concentration of furanocoumarins and angular-type dihydropyranocoumarins were found to range between 10.05 and 68.12 mg/kg, and 2.18 and 5.47 mg/kg, respectively, and the concentration of coumarin was below 20 mg/kg adding up to an average of 60 mg/kg total coumarins.

TABLE 3 Analyses of coumarins in five batches of the NF.

Parameter (mg/kg)	Batch number #1	#2	#3	#4	#5	Method of analysis
Coumarin	< 20	< 20	< 20	< 20	< 20	LC-MS/MS
Furanocoumarins						
Bergapten	2.96	1.15	3.88	7.47	3.79	LC-MS/MS (internal)
Xanthotoxin	2.22	< 1**	2.42	4.67	2.51	
Isopimpinellin	2.99	1.07	3.37	7.00	3.22	
Oxypeucedanin	4.94	2.52	9.12	16.31	8.75	
Oxypeucedanin hydrate	13.03	5.31	14.15	22.52	12.30	
Isoimperatorin	2.06	< 1**	2.87	6.86	2.87	
Angular furanocoumarins						
Angelicin	1.82	< 1**	1.83	3.29	1.73	LC-MS/MS (internal)
Sum of furanocoumarins	30.02	10.05	37.64	68.12	35.17	
Angular-type dihydropyranocoumarins						
Laserpitin	0.84	1.12	1.37	1.48	1.13	HPLC-UV (internal)
Isolaserpitin	1.02	1.49	2.55	3.12	1.85	
Selinidin	0.32	0.46	0.72	0.87	0.55	
Sum of angular-type dihydropyranocoumarins	2.18	3.07	4.64	5.47	3.53	
Total coumarins*	32.20	13.12	42.28	73.59	38.70	

Abbreviations: HPLC-UV, high-performance liquid chromatography- ultraviolet detection; LC-MS/MS, liquid chromatography-Tandem mass spectrometry.
*Total coumarins: Sum of furanocoumarins and angular-type dihydropyranocoumarins.
**LOQ.

Analytical data for chemical and microbiological parameters were provided for the five independently produced batches of the NF (Table 4). All analysed batches were found within the specification limits.

TABLE 4 Analyses of chemical and microbiological contaminants in five batches of the NF.

Parameter	Batch number #1	#2	#3	#4	#5	Method of analysis
Heavy metals						
Lead (mg/kg)	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*	ICP-MS (internal)
Arsenic (mg/kg)	< 0.1*	< 0.1*	< 0.1*	< 0.1*	< 0.1*	ICP-MS (internal)
Mercury (mg/kg)	< 0.005*	< 0.005*	< 0.005*	< 0.005*	< 0.005*	ICP-MS (internal)
Cadmium (mg/kg)	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	ICP-MS (internal)
Microbiological parameters						
Total viable aerobic count (CFU/g)	100	< 100*	< 100*	200	200	Spiral plate technique; ISO 4833-2:2014-05

(Continues)

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TABLE 4 (Continued)

Parameter	Batch number #1	#2	#3	#4	#5	Method of analysis
Yeast/mould (CFU/g)	< 100*	< 100*	< 100*	< 100*	< 100*	Plate counting method; ISO 21527-2:2008
Salmonella s pp. (/25g)	Not detected	Not detected	Not detected	Not detected	Not detected	Detection method; ISO 6579:2017-07
Beta-glucuronidase-positive *Escherichia coli* (CFU/g)	< 10*	< 10*	< 10*	< 10*	< 10*	Plate counting method; ISO 16649-2:2009-12
Coliforms (CFU/g)	< 10*	< 10*	< 10*	< 10*	< 10*	Plate counting method; ISO 4832:2006-02

Abbreviations: CFU, colony forming units; ISO, International Organization for Standardization; ICP-MS, Inductively coupled plasma mass spectrometry.
*LOQ.

Moreover, the concentration of 12 carotenoids[4] was measured in the five batches of the NF and the sum of carotenoids measured found to be in a range of 0.04–0.16 μg/g.

Pesticides (350 substances) were also analysed in the five batches of the NF and were found below the limit of quantification in all five batches analysed (i.e. LOQ ≤ 0.01 mg/kg).

In addition to the NF, the applicant provided proximate analyses and analytical data on the chalcone content for six independently produced batches of the product as it is intended to be placed on the market containing ashitaba sap powder (Tables 5 and 6). In these batches, the applicant highlighted that the percentage of chalcones, expressed as the sum of xanthoangelol and 4-hydroxyderricin, was at least 8%.

TABLE 5 Batch to batch proximate analysis of six batches of the product as it is intended to be placed on the market containing ashitaba sap powder.

Parameter (%)	Batch number #6	#7	#8	#9	#10	#11	Method of analysis
Water	0.6	3.0	2.9	1.9	3.5	4.9	Gravimetry
Carbohydrates**	88.8	83.9	83.7	85	81.7	80.1	Calculation
Fat*	9.4	12.0	12.4	12	13.3	13.9	Gravimetry
Protein (as-is, $N \times 6.25$)	0.5	0.6	0.4	0.6	0.9	0.8	Titrimetry (Kjeldahl method)
Ash	0.7	0.6	0.6	0.5	0.6	0.3	Gravimetry
Total	100	100.1	100	100	100	100	

*Chalcones are expected to be partially included in the fat measurements; gravimetric method used is unspecific.
**Around 70% of the carbohydrates correspond to linear and branched cyclodextrin content of the NF and the rest 6.7%–8.4% is attributed to the uncharacterised proportion of ashitaba sap.

TABLE 6 Analyses of chalcones in six batches of the product as it is intended to be placed on the market containing ashitaba sap powder.

Parameter (%)	Batch number #9	#10	#11	#12	#13	#14	Method of analysis
Xanthoangelol*	5.49	5.31	5.35	5.19	5.54	5.44	HPLC (internal method)
4-Hydroxyderricin*	2.98	3.15	3.10	3.06	3.16	3.12	HPLC (internal method)
Total Chalcone*	8.47	8.46	8.45	8.25	8.70	8.56	Sum of xanthoangelol and 4-hydroxyderricin

Abbreviation: HPLC, high-performance liquid chromatography.
*Expressed on whole weight.

The applicant provided analytical data on the coumarin content in three batches of the product as it is intended to be placed on the market containing ashitaba sap powder, in particular amounts of three furanocoumarins (psoralen, isopimpinellin, bergapten) and three pyranocoumarins (laserpitin, isolaserpitin, selindin) (Table 7). The product as it is intended to be placed on the market contains on average 38,615 mg/kg coumarins expressed as the sum of the aforementioned measured coumarins.

[4]Carotenoids analysed by HPLC-VIS/DAD: alpha-carotene; *cis*-beta-carotene; *trans*-beta-carotene; total beta-carotene; alpha-cryptoxanthin; (all-E)-β-cryptoxanthin; gamma-carotene; *cis*-lycopene; trans-lycopene; total lycopene; lutein; zeaxanthin.

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TABLE 7 Analyses of coumarins in three batches of the product as it is intended to be placed on the market containing ashitaba sap powder.

Parameter (mg/kg)	Batch number #15	#16	#17	Method of analysis
Furanocoumarins				
Bergapten	260	211	205	HPLC-UV (internal)
Xanthotoxin (psoralen)	795	774	711	
Isopimpinellin	937	928	782	
Sum of furanocoumarins	1992	1913	1698	
Angular-type dihydropyranocoumarins				
Laserpitin	13,059	15,560	13,599	HPLC-UV (internal)
Isolaserpitin	14,404	19,534	15,193	
Selinidin	5655	7544	5694	
Sum of angular-type dihydropyranocoumarins	33,118	42,638	34,486	
Total coumarins*	35,110	44,551	36,184	

Abbreviation: HPLC-UV, high-performance liquid chromatography-ultraviolet detection.
*Total coumarins: Sum of furanocoumarins and angular-type dihydropyranocoumarins.

Analytical data for chemical and microbiological parameters were provided for five independently produced batches of the product as it is intended to be placed on the market (Table 8).

TABLE 8 Analyses of chemical and microbiological contaminants in five batches of the product as it is intended to be placed on the market containing ashitaba sap powder.

Parameter (%)	Batch number #9	#10	#11	#12	#13	#14	Method of analysis
Heavy metals							
Lead (mg/kg)	< 0.01*	0.012	< 0.01*	< 0.01*	0.016	0.011	ICP-MS
Arsenic (mg/kg)	< 0.1*	< 0.1*	< 0.1*	< 0.1*	< 0.1*	< 0.1*	ICP-MS
Mercury (mg/kg)	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	< 0.01*	ICP-MS
Cadmium (mg/kg)	< 0.004*	< 0.004*	< 0.004*	< 0.004*	< 0.004*	< 0.004*	ICP-MS
Microbiological parameters							
Total viable aerobic count (CFU/g)	< 300*	< 300*	< 300*	< 300*	< 300*	< 300*	Spiral plate count technique; U.S. FDA BAM (Chapter 3)
Yeast & mould (CFU/g)	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	Plate counting method; U.S. FDA BAM (Chapter 18)
Salmonella spp. (per 25 g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Detection method; AOAC Sec. 967.26
Escherichia coli (per g)	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected	Plate counting method; U.S. FDA BAM (Chapter 4a)
Coliforms (CFU/g)	< 10*	< 10*	< 10*	< 10*	< 10*	< 10*	Plate counting method; ISO 4832:1991

Abbreviations: AOAC, Association of Official Analytical Chemists; BAM, bacteriological analytical manual; CFU, colony forming units; ICP-MS, inductively coupled plasma mass spectrometry; U.S. FDA, United States Food and Drug Administration.
*LOQ.

Analysis of carotenoids including α-carotene, β-carotene, lutein, zeaxanthin and lycopene was also performed in one batch of the product as it is intended to be placed on the market and no carotenoid was found above the limits of quantification (α-carotene, β-carotene LOQ: 6 μg/100 g; lutein, zeaxanthin and lycopene LOQ: 0.02 mg/100 g). Polycyclic aromatic hydrocarbons (PAH) were also analysed in three batches of the product as it is intended to be placed on the market and were found below the limit of quantification in all three batches analysed (i.e. LOQ ≤ 10 μg/kg).

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

3.4.1 | Stability

The applicant focused stability testing on the stability of chalcones in the product as it is intended to be placed on the market. The applicant proposed a shelf-life for the product as it is intended to be placed on the market of 3 years under airtight packaging in a cool environment and thus tested the degradation of chalcones in five independently produced batches at 25°C. The chalcone content measurements were conducted at different time points for each batch spanning from 6 to 52 months. No significant changes were observed in the concentration of chalcones over the period tested.

The applicant tested also the stability of the isolated chalcones xanthoangelol and 4-hydroxyderricin from the NF over a 30-month period at both 5°C and room temperature (isolation procedure and number of samples not reported) and concluded that chalcones were found to be stable when stored at 5°C or at room temperature for 30 months (ambient humidity), respectively.

The stability of the product as it is intended to be placed on the market was also tested under various conditions of temperature (50°C, 100°C and 120°C), and pH (pH 3, pH 7 ad pH 10) for 60 min. Based on the results, chalcones are relatively stable at varying pH levels when stored at 50°C for up to 60 min, whereas at temperatures higher than 50°C under neutral (pH 7) or alkaline (pH 10) conditions, a decrease in the concentration of chalcones was observed. Moreover, the decrease in the concentration of chalcones was enhanced by an increase in pH when exposed to temperatures greater than 50°C.

The applicant also provided stability data on microbiological parameters of the product as it is intended to be placed on the market. Analytical testing was performed on five independent batches between 40 and 95 months, with each batch being tested at a different time point during this period. None of the batches exceeded the LOQ of each testing method.

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

3.5 | Specifications

The specifications of the NF as proposed by the applicant are indicated in Table 9.

The applicant noted that the NF will only be used as a powder, containing around 30% of ashitaba sap powder and 70% cyclodextrins standardised for the chalcone content (at least 8%).

TABLE 9 Specifications of the NF.

Description: The NF is the sap, which is a viscous yellow liquid, from the stems of *Angelica keiskei* (ashitaba) plants	
Source: *Angelica keiskei*, Apiaceae	
Parameter	**Specification**
Chalcones (xanthoangelol +4-hydroxyderricin)	1%–2.25%, w/v
Water	90%–95%
Carbohydrates	5%–7.5%
Fat	0.1%–0.3%
Protein	0.15%–0.45%
Sum of angular-type dihydropyranocoumarins	< 10 mg/kg
Sum of furanocoumarins	< 100 mg/kg
Heavy metals	
Lead	≤ 0.1 mg/kg
Arsenic	≤ 0.3 mg/kg
Mercury	≤ 0.1 mg/kg
Cadmium	≤ 1 mg/kg
Microbiological	
Total viable aerobic count	≤ 1000 CFU/g
Yeast/mould	≤ 100 CFU/g
Salmonella	Not detected in 25 g
Escherichia coli	Not detected in 10 g
Coliforms	≤ 30 CFU/g

Abbreviations: CFU, Colony forming units; w/v, weight per volume.

The Panel considers that the information provided on the specifications of the NF is sufficient.

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

3.6.1 | History of use of the source

Angelica keiskei is a perennial herb native to Japan and is cultivated in areas of Eastern Asia, including Japan and Korea (USDA, 2009). The ashitaba plant is consumed in Japan as a vegetable and juice (Ohkura et al., 2011; Ohnogi Hiromi et al., 2012). In addition, fresh ashitaba leaves and dried powder can be added in 'soba' noodles and ice cream. Ashitaba (parts of the plant not specified) can also be found in products such as ashitaba tea, cakes, powder, candy and a Japanese confectionary called yatshuhashi (Maronpot, 2015). The history of use of this plant is corroborated by its presence in the list of the imported food products from Japan in the EU on which special conditions were imposed in relation to the import of feed and food originating from Japan following the Fukushima nuclear power station accident (Implementing Regulation (EU) No 996/2012).[5]

3.6.2 | History of use of the product as it is intended to be placed on the market

The applicant noted that the product as it is intended to be placed on the market has been marketed under the brand name 'ChalSap-P8' as a food in Japan. Following an EFSA request to provide the number of years, the 'ChalSap-P8' has been on the market in Japan; the applicant responded that it has been on the Japanese market for over 22 years. The applicant reported that they sold roughly 1000 kg of the product as it is intended to be placed on the market in Japan, between August 2017 and July 2018.

3.7 | Proposed uses and use levels and anticipated intake

3.7.1 | Target population

The NF is intended for use by adults, excluding pregnant and lactating women.

3.7.2 | Proposed uses and use levels

The applicant intends to market the NF in the EU for use in food supplements as a freeze-dried product consisting of 30% ashitaba sap powder and 70% cyclodextrins (the product as it is intended to be placed on the market). The maximum use level proposed by the applicant for the product as it is intended to be placed on the market is 200 mg per day (freeze-dried product), corresponding to a maximum use level of 780 mg of the NF per day (containing 90–95% of water).[6] The applicant intends to sell a freeze-dried product consisting of 30% ashitaba sap powder and 70% cyclodextrins in the form of capsules, tablets and powder.

3.7.3 | Anticipated intake of the NF

The anticipated intake of the product as it is intended to be placed on the market (freeze-dried product) reaches 2.86 mg/kg bw per day and corresponds to levels up to 11.1 mg/kg bw per day of the NF (containing ~ 92.9% of water) for an adult, considering the default weight for an adult of 70 kg (EFSA Scientific Committee, 2012).

3.7.4 | Anticipated intake of other substances than nutrients

Based on the specifications of the NF (Table 9), the intake of chalcones is expected to range from 7.80 to 17.6 mg per day and the intake of furanocoumarins is estimated up to 0.078 mg per day.

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

The applicant presented a study investigating the bioavailability of the most abundant chalcone components of the NF, i.e. 4-hydroxyderricin and xanthoangelol, in mice. Male ICR mice were administered either an ethyl acetate extract prepared

[5]Commission Implementing Regulation (EU) No 996/2012 of 26 October 2012 imposing special conditions governing the import of feed and food originating in or consigned from Japan following the accident at the Fukushima nuclear power station and repealing Implementing Regulation (EU) No 284/2012 Text with EEA relevance. OJ L 299, 27.10.2012, p. 31–41 (Date of end of validity: 31 March 2014).

[6]The conversion of the maximum use levels of the product as it is intended to be placed on the market to the NF was performed by the applicant based on the chalcone content in the two formulations.

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from the product as it is intended to be placed on the market (referred to as ashitaba EA extract) containing 145 µg/mg (dry weight) of 4-hydroxyderricin and 173 µg/mg (dry weight) of xanthoangelol (corresponding to 31% chalcones: 17% xanthoangelol and 14% 4- hydroxyderricin) or the purified chalcones separately (Nakamura et al., 2012). The ashitaba EA extract was administered orally in single doses of 50–500 mg/kg bw, whereas the doses of 4-hydroxyderricin and xanthoangelol given individually were equivalent to those in the 200 mg/kg bw dose of the ashitaba EA extract (Nakamura et al., 2012). The dilution solvent used was 5% polysorbate 80 (Nakamura et al., 2012).

The concentrations of 4-hydroxyderricin and xanthoangelol were measured in blood, urine and faeces collected at 0–24 h after administration. Both chalcones (aglycone and glucuronate/sulfate conjugated forms) were detected into the plasma, with time-to-maximum plasma concentrations at 2 h for free and conjugated 4-hydroxyderricin, and at 0.5 h for aglycone and 1 h for conjugated xanthoangelol, after oral administration of the ashitaba EA extract. The time-to-maximum plasma concentrations dropped to 1 and 0.5 h for 4-hydroxyderricin and xanthoangelol, respectively, after oral administration of the individual chalcones. Despite the structural similarity of the chalcones under study, a greater bioavailability of 4-hydroxyderricin was evidenced by the four fold higher total plasma concentration of 4-hydroxyderricin compared to xanthoangelol at 24 h (expressed both as aglycon and sum of aglycon and conjugated forms). The tissue distribution of 4-hydroxyderricin and xanthoangelol was measured in the liver, kidney, spleen, muscle and white adipose tissues (perirenal, epididymal and mesenteric fat) 2 h after administration of 200 mg/kg bw of the ashitaba EA extract. The aglycone form of both compounds was predominantly detected in all the tissues examined except for mesenteric adipose tissue. The total chalcones (aglycon and conjugated) exhibited differential distribution, with 4-hydroxyderricin present primarily in the adipose tissues and xanthoangelol present primarily in the liver and mesenteric fat. Excretion of both chalcones was mostly via the urinary system between 2 and 4 h after oral administration of the ashitaba EA extract in their aglycone and conjugated forms. Both chalcones were also almost exclusively detected in their aglycone forms in the faecal samples collected 24 h after oral administration of the ashitaba EA extract. The authors noted that the bioavailability of polyphenols is higher in humans than that in rodents and highlighted the need of human studies to investigate further the bioavailability of the chalcones (Nakamura et al., 2012).

3.9 | Nutritional information

The NF is predominantly composed of water (90–95%), and in smaller proportion of fat (~ 0.2%), protein (~ 0.3%) and ash (~ 0.2%). The Panel considers that taking into account the composition of the NF and the proposed consumption (780 mg/day), the NF is not nutritionally disadvantageous.

3.10 | Toxicological information

The applicant provided a battery of toxicological studies conducted with the NF and the product as it is intended to be placed on the market containing ashitaba sap powder, which were claimed proprietary and confidential by the applicant and are listed in Table 10.

TABLE 10 List of toxicological studies with the NF and the product as it is intended to be placed on the market containing ashitaba sap powder.

Test substance	Reference	Type of study	Test system	Concentration/dose
Genotoxicity				
Product as it is intended to be placed on the market	Study No 44005–16 (Krul, 2002)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium: TA 1535, TA 1537, TA 98, TA 100; *Escherichia coli* WP2 uvrA	Up to 1000 µg/plate (absence and presence of S9 mix)
Product as it is intended to be placed on the market	Study No. 5002/02 (de Vogel, 2003)	In vitro mammalian chromosomal aberration test (GLP, OECD 473)	CHO K-1 cell line	Up to 250 µg/mL (absence and presence of S9 mix)
Product as it is intended to be placed on the market (Lot no # 9)	Study No. 282452 (Cardoso & Licollari, 2014)	In vivo mammalian erythrocyte micronucleus test (GLP compliant, OECD TG 474)	Swiss Albino (CD-1) mice	Up to 2000 mg/kg bw per day (oral gavage)
Ashitaba sap (NF) (Lot no # 5)	Study No. ARL/G/22/PT1253 (Joshi, 2023a)	Bacterial reverse mutation test (GLP, OECD TG 471)	*Salmonella* Typhimurium: TA 102, TA 1535, TA 1537, TA 98, TA 100	Up to 98.81 mg/plate (absence and presence of S9 mix)
Ashitaba sap (NF) (Lot no # 5)	Study No. ARL/G/22/PT1254 (Joshi, 2023b)	In vitro mammalian cell micronucleus test (GLP, OECD TG 487)	Human peripheral blood lymphocytes	Up to 243.84 mg/mL (absence and presence of S9 mix)
Acute and subchronic toxicity				
Product as it is intended to be placed on the market	Study No. 4410/06 (Prinsen, 2002)	Acute oral toxicity study-Acute toxic class method (GLP, OECD TG 423, limit test)	Wistar rats	2000 mg/kg bw per day

TABLE 10 (Continued)

Test substance	Reference	Type of study	Test system	Concentration/dose
Product as it is intended to be placed on the market	Study No. B-5530 (Oda, 2006)	90-day repeated dose oral toxicity study (GLP, according to Japanese Guidelines)	Sprague Dawley rats	Up to 1000 mg/kg bw per day
Product as it is intended to be placed on the market	Study No. 12-028-6 (Kukulinski, 2013)	90-day repeated dose oral toxicity study (no guideline study)	Sprague Dawley rats	600 mg/kg bw per day
High-fat diet containing the product as it is intended to be placed on the market	(Nagata et al., 2007)	28-day ad libitum fed study (according to guidelines of the Incorporated Administrative Agency, National institute of Health and Nutrition)	Wistar rats	Up to 17,000 mg/kg bw per day

Abbreviations: CHO, Chinese hamster ovary; GLP, Good Laboratory Practice; NF, Novel Food; OECD, Organization for Economic Cooperation and Development; TG, Test guidelines.

3.10.1 | Genotoxicity

The applicant initially presented genotoxicity assays performed with the product as it is intended to be placed on the market, and upon request from EFSA, the applicant provided a bacterial reverse mutation test and an in vitro mammalian cell micronucleus test performed with the NF.

3.10.1.1 | *Genotoxicity of the product as it is intended to be placed on the market*

The potential genotoxicity of the product as it is intended to be placed on the market was examined based on the results obtained from a bacterial reverse mutation test (Krul, 2002, unpublished study report), an in vitro mammalian chromosomal aberration test (de Vogel, 2003, unpublished study report) and an in vivo micronucleus test (Cardoso & Licollari, 2014, unpublished study report).

The bacterial reverse mutation test (Krul, 2002, unpublished study report) was performed using histidine dependent auxotrophic mutants of *Salmonella* Typhimurium, strains TA1535, TA1537, TA98 and TA100, and a tryptophan-dependent mutant of *E. coli*, strain WP2 uvrA. The product as it is intended to be placed on the market was diluted in DMSO and five different concentrations of test substance from 12.3, 37, 111, 333 and to 1000 μg/plate (corresponding to 0.3–24 μg chalcones/plate) were tested both in the presence and absence of liver microsomal fractions (S9). The product as it is intended to be placed on the market was found toxic to *S.* Typhimurium TA 100 at doses above and including 111 μg/plate both with and without metabolic activation (S9) whereas no mutagenicity was evidenced based on the mean number of revertant colonies appearing in the test plates compared to the background spontaneous reversion rate observed with the negative control. Therefore, the product as it is intended to be placed on the market did not exhibit mutagenicity at concentrations up to 1000 μg/plate, in the absence or presence of metabolic activation.

The chromosomal aberration test (Krul, 2002, unpublished study report) was conducted with the product as it is intended to be placed on the market in Chinese hamster ovary cells [CHO (K-1) line] at final concentrations in the culture media ranged from 5 to 2500 μg/mL following two treatment regimens. The product as it is intended to be placed on the market was found toxic at high concentrations (2500, 1250, 625 and 313 μg/mL), and thus, doses up to 300 μg/mL (corresponding to 7.2 μg chalcones/mL) were studied. Following a continuous treatment at 4 and 8 h without metabolic activation (S9) and harvesting at 18 h and a 4 h treatment with metabolic activation (S9) and harvesting at 18 h, no statistically significant increase was observed in the number of aberrant cells at all non-cytotoxic concentrations of the NF and time points analysed. Continuous treatment of cells at 18 and 32 h without metabolic activation (S9) did not induce a statistically significant increase in aberrant cell counts. However, a short-term treatment at 4 h with metabolic activation (S9) and harvesting at 18 and 32 h showed a significant increase in aberrant cells at 32 h for the 300 μg/mL dose. This positive response (i.e. 11.5% of treated cells at 300 μg/mL showing aberrations vs. 0% of the negative control) was attributed to the severe transient cytotoxicity observed during the 4-h treatment period and considered non-relevant.

The in vivo micronucleus test (Cardoso & Licollari, 2014, unpublished study report) was carried out in Swiss Albino (CD-1) mice. Fourteen male mice were administered with the product as it is intended to be placed on the market by oral gavage at a single limit dose of 2000 mg/kg bw (corresponding to 43.2 mg chalcones/kg bw). A vehicle control group [methylcellulose (1%)] as well as a positive control group [cyclophosphamide (70 mg/kg)] were included in the study. Seven mice in each group were terminated 24- and 36-hour post-dosing, and harvested bone marrow was stained. Two thousand polychromatic erythrocytes (PCEs) per animal were scored for the presence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was determined per 500 cells and the number of micronuclei in normochromatic erythrocytes (NCEs) was also determined. There was no effect on body weight observed during the study. The product as it is intended to be placed on the market did not induce any micronucleated PCEs, as evidenced by the results of micronuclei scoring, showing a similar number of PCEs with micronuclei between the product as it is intended to be placed on the market group

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and the negative control group. However, this assay did not provide evidence of bone marrow exposure to the product as it is intended to be placed on the market since the PCE/NCE ratio (per total of 500 cells) at the limit dose (2000 mg/kg bw) was unchanged compared to vehicle control.

3.10.1.2 | *Genotoxicity of the NF*

Following an EFSA request, the applicant performed a bacterial reverse mutation test and an in vitro mammalian cell micronucleus test with the NF. As the NF contains furanocoumarins which act as DNA cross linkers in the presence of UVA light, the applicant was requested to perform these tests under UV protection. Protection from UV exposure was ensured for the entire duration of both studies.

The potential mutagenicity of the NF was assessed in a bacterial reverse mutation test (Joshi, 2023a, unpublished report) using *Salmonella* Typhimurium test strains TA98, TA100, TA102, TA1535 and TA1537 in the absence and presence of metabolic activation mix (S9), using the plate-incorporation and pre-incubation methods. 2-Aminoanthracene was used as the sole positive control for the S9 mix activity. After preliminary experiment, doses of the NF at 0.99, 2.96, 9.88, 29.64 and 98.81 mg/plate were selected for the main test (the highest concentration was the highest feasible concentration based on solubility in the vehicle). DMSO was chosen as the dilution solvent and comprised a range of 0%–3.69% (v/v) in the bacterial plating mix. The above-mentioned concentrations of the NF were selected to provide exposure to chalcones at concentrations between 0.0203 and 2.026 mg/plate. Neither cytotoxicity nor precipitation of the test article was observed at any concentration in either the preliminary or the main test. Treatment with the NF did not result in increases in the number of revertant colonies as compared to the negative controls at any concentration in both tests either in the presence or absence of S9. Thus, the NF was determined to be non-mutagenic in this test at concentrations up to 98.81 mg/plate.

The genotoxic potential of the NF was further investigated in an in vitro mammalian cell micronucleus test (Joshi, 2023b, unpublished report) conducted in human peripheral blood lymphocytes. The highest concentration of the NF was selected to be 243.84 mg/mL based on solubility and precipitation of the NF and the results of a preliminary cytotoxicity assay using the Cytokinesis Block Proliferation Index (CBPI). The NF was diluted in karyotyping medium consisting of basal medium and phytohaemagglutinin-M. The highest concentration of the NF used in the assay corresponded to a maximum concentration of chalcones at 5 mg/mL. The preliminary assay showed no cytotoxicity at the any of the six test concentrations (range 7.62–243.84 mg/mL) of the NF compared to the vehicle control. It encompassed a short-term exposure (3 h) in the presence or absence of metabolic activation and a long-term exposure (28 h) in the absence of metabolic activation. Based on the results of the preliminary cytotoxicity assay, three concentrations of the NF namely at 15.24, 60.96 and 243.84 mg/mL (chalcone range 0.3125–5 mg/mL) were used for the main experiment including a 3-h exposure with or without metabolic activation, and a 28-h exposure without metabolic activation. There were no differences observed in the number and percentage of binucleate cells containing micronuclei at any of the concentrations of the NF analysed as compared to the negative control. The NF was therefore determined to be non-clastogenic and non-aneugenic in cultured human peripheral blood lymphocytes.

Linear type furanocoumarins including bergapten, xanthotoxin, isopimpinellin, oxypeucedanin, oxypeucedanin hydrate and isoimperatorin have the potential to form DNA cross-links after UVA exposure. However, due to the pre-systemic metabolism, furanocoumarins are unlikely to reach the skin at the concentrations present in the NF, given the low oral exposure to furocoumarins (maximum 78 μg per day).

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

3.10.2 | Acute and subacute toxicity

The applicant provided an acute oral toxicity study (Prinsen, 2002, unpublished report) performed with the product as it is intended to be placed on the market in outbred Wistar rats (3 animals/sex). The limit dose (2000 mg/kg bw) was administered by oral gavage after overnight fasting. The animals were observed for 14 days for clinical signs, and macroscopic examination was performed at the end of the study. Body weight was also recorded at days 0, 3, 7 and 14 of the study. During the 14-day observation period, no abnormal clinical signs were observed. By the end of the 14-day observation period, all rats had gained weight and had shown no treatment-related macroscopic alterations at study termination and necropsy. The applicant also referred to a 28-day study performed in male Wistar rats receiving high fat diets containing the product as it is intended to be placed on the market (3 groups: 170, 1700, 17,000 mg/kg bw per day) (Nagata et al., 2007). Overall, there were no significant differences in body weight, epididymal adipose tissue weight, serum cholesterol, liver lipid concentrations or other biochemical profiles in the serum. Additionally, no significant pathological findings in the liver or kidney were observed.

3.10.3 | Subchronic toxicity

The applicant provided a 90-day repeated dose oral toxicity study (Oda, 2006) conducted in Sprague Dawley SPF rats, starting at 6 weeks of age (12/sex/dose group and control group). The product as it is intended to be placed on the market was diluted in olive oil and administered to the animals by oral gavage at doses of 0, 100, 300 and 1000 mg/kg bw per day.

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In the study, two incidental early deaths (one control male and one 300 mg/kg bw per day ashitaba sap-treated male) occurred. All remaining animals were without abnormal clinical signs throughout the study period. In addition, no differences in body weight, food consumption, ophthalmological examination or urinalysis between control and treated groups were reported.

The haematological examination, carried out at the end of the treatment, revealed several statistically significant findings (Table 11). Haemoglobin was found reduced in males administered with the test substance at highest dose (1000 mg/kg bw per day). Moreover, statistically significant decreases in platelet counts were present in high-dose male and female rats along with increased coagulation parameters in both sexes. In particular, prothrombin time (PT) in male and female rats was statistically significantly prolonged in the 300 and 1000 mg/kg bw per day groups compared to the control group, and activated partial thromboplastin time (APTT) was increased in high-dose (1000 mg/kg bw per day) males.

TABLE 11 Selected haematology findings in Sprague Dawley rats (90-day oral study, values expressed as means ± SD). Statistically significant findings are indicated in bold.

Dose (mg/kg bw per day)	0 (*n* = 11)	100 (*n* = 12)	300 (*n* = 11)	1000 (*n* = 12)
Parameter	**Males**			
Haemoglobin (g/dL)	16.4 ± 0.3	16.3 ± 0.5	16.3 ± 0.4	**15.8 ± 0.3****
Platelets (×10^4/µL)	105.1 ± 9.6	102.1 ± 5.5	98.3 ± 8.4	**93.2 ± 10.0****
Prothrombin time (s)	15.4 ± 1.6	16.6 ± 2.2	**21.4 ± 3.3****	**24.5 ± 4.5****
APTT (s)	24.2 ± 3.2	23.7 ± 2.2	25.3 ± 2.7	**30.7 ± 5.8****
Dose (mg/kg bw per day)	**0 (*n* = 12)**	**100 (*n* = 12)**	**300 (*n* = 12)**	**1000 (*n* = 12)**
Parameter	**Females**			
Platelets (10^4/µL)	100.4 ± 10.6	95.1 ± 8.9	95.7 ± 8.1	**80.8 ± 6.5****
Prothrombin time (s)	12.5 ± 0.6	12.8 ± 0.5	**13.4 ± 0.6****	**13.2 ± 0.4****

**$p \leq 0.01$, as reported by Maronpot (2015).

Clinical chemistry parameters were also examined and statistically significant increases in serum alkaline phosphatase (ALP), total cholesterol and phospholipids were observed in high-dose (1000 mg/kg per day) male and female rats (Table 12). Elevated serum triglyceride, blood urea nitrogen (BUN) values and significantly reduced sodium and potassium values were reported only in high-dose males. A significant increase in serum aspartate aminotransferase (AST) was seen only in high-dose females. In the protein fractions, statistically significant changes were observed in some globulin isoforms (α_1 Globulin, α_2 Globulin & β Globulin) in 300 and 1000 mg/kg bw per day groups of males and females.

TABLE 12 Selected findings in serum clinical chemistry in Sprague Dawley rats (90-day oral study, values expressed as means ± SD). Statistically significant findings are indicated in bold.

Dose (mg/kg bw per day)	0 (*n* = 11)	100 (*n* = 12)	300 (*n* = 11)	1000 (*n* = 12)
Parameter	**Males**			
ALP (IU/L)	339 ± 71	345 ± 96	363 ± 122	**496 ± 131****
Total cholesterol (mg/dL)	68 ± 18	79 ± 17	76 ± 13	**125 ± 42****
Triglycerides (mg/dL)	49 ± 27	92 ± 46	97 ± 47	**144 ± 62****
Phospholipids (mg/dL)	113 ± 21	136 ± 17	139 ± 20	**225 ± 80****
BUN	10 ± 1	10 ± 1	11 ± 1	**12 ± 1***
Na (mmol/L)	143 ± 1	144 ± 1	143 ± 1	**142 ± 1***
K (mmol/L)	4.8 ± 0.4	4.5 ± 0.3	4.6 ± 0.4	**4.4 ± 0.3***
Albumin/globulin ratio	0.73 ± 0.06	0.74 ± 0.06	0.79 ± 0.06	0.78 ± 0.05
Albumin (%)	42.2 ± 1.9	42.4 ± 2.0	43.9 ± 2.0	43.8. ± 1.6
α_1 Globulin (%)	22.0 ± 1.8	21.1 ± 2.2	**18.6 ± 1.2****	**16.7 ± 1.2***
α_2 Globulin (%)	10.0 ± 0.7	10.6 ± 0.7	10.7 ± 0.6	**11.6 ± 0.7****
β Globulin (%)	18.5 ± 1.2	18.9 ± 1.1	**20.0 ± 1.1***	**21.7 ± 1.0****
Dose (mg/kg bw per day)	**0 (*n* = 12)**	**100 (*n* = 12)**	**300 (*n* = 12)**	**1000 (*n* = 12)**
Parameter	**Females**			
AST (IU/L)	64 ± 30	57 ± 11	53 ± 6	**84 ± 43***
ALP (IU/L)	146 ± 35	194 ± 49	181 ± 30	**274 ± 91****
Total cholesterol (mg/dL)	74 ± 19	82 ± 13	80 ± 10	**109 ± 25****

(Continues)

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TABLE 12 (Continued)

Dose (mg/kg bw per day)	0 ($n=11$)	100 ($n=12$)	300 ($n=11$)	1000 ($n=12$)
Phospholipids (mg/dL)	144 ± 31	161 ± 18	152 ± 16	**182 ± 32****
Albumin/globulin ratio	0.85 ± 0.09	0.91 ± 0.07	0.88 ± 0.06	0.87 ± 0.08
Albumin (%)	46.0 ± 2.6	47.5 ± 2.0	46.7 ± 1.7	46.5 ± 2.4
α_1 Globulin (%)	18.8 ± 1.5	17.7 ± 1.2	**16.9 ± 1.3***	**13.7 ± 2.2****
α_2 Globulin (%)	8.9 ± 0.6	8.7 ± 0.7	9.1 ± 0.7	**10.1 ± 1.0****
β Globulin (%)	17.1 ± 1.9	16.9 ± 1.2	18.3 ± 1.1	**21.4 ± 1.3****

*$p \le 0.05$, as reported by Maronpot (2015). **$p \le 0.01$, as reported by Maronpot (2015).

Determinations of the weights of selected organs and tissues at necropsy were performed at the end of the study. Increased relative kidney weight was observed in high-dose males (1000 mg/kg bw per day). Changes in absolute brain and adrenal weight were observed in males at 300 and 1000 mg/kg bw per day (Table 13). An increase in the relative heart weight was also observed in 300 mg/kg bw per day males. No statistical significant changes were observed in absolute and relative liver weights in both sexes.

TABLE 13 Selected findings in organ weights in Sprague Dawley rats (90-day oral study, values expressed as means ± SD). Statistically significant findings are indicated in bold.

Dose (mg/kg bw per day)	0 ($n=11$)	100 ($n=12$)	300 ($n=11$)	1000 ($n=12$)
Absolute weight	**Males**			
Brain weight (g)	2.17 ± 0.08	2.15 ± 0.08	**2.07 ± 0.07****	**2.10 ± 0.05***
Adrenal (mg)	61 ± 5	60 ± 10	**51 ± 8****	**53 ± 7***
Relative weight				
Heart (g/100 g BW)	0.26 ± 0.02	0.26 ± 0.02	**0.29 ± 0.02****	0.27 ± 0.02
Kidney (g/100 g BW)	0.59 ± 0.04	0.59 ± 0.04	0.62 ± 0.02	**0.65 ± 0.06***

*$p \le 0.05$, as reported by Maronpot (2015). **$p \le 0.01$, as reported by Maronpot (2015).

A number of histopathological findings observed in the subchronic study by Oda (2006) were also later peer-reviewed independently by Seely (2012) and in a study published by Maronpot (2015) (Table 14). Minimal increased vacuolation was observed in adrenals at 1000 mg/kg bw per day group in males and females. There was minimal or mild thickening of limiting ridge in the stomach in 1000 mg/kg bw per day males and females. In the caecum, minimal cell infiltration in lamina propria was seen in control males, 100, 300, 1000 mg/kg bw per day males, and control and 1000 mg/kg bw per day females.

In the small intestine, dilated lacteals (jejunum) were observed in both sexes for the high-dose group (males and females). In the kidney, histopathological changes were observed in male rats in a dose-dependent manner at low-dose, mid-dose and high-dose. The Panel notes that kidney histopathology findings and globulins may point to kidney toxicity. The presence of alpha 2u-globulin in intracytoplasmic hyaline droplets in kidney cells are difficult to assess by typical haematoxylin and eosin (H&E) staining. As a result, EFSA requested experimental data that demonstrate accumulation of alpha 2u-globulin in kidney cells, which might be confirmed by immunodetection of alpha 2u-globulin via immunohistochemistry. In response to EFSA's request, the applicant was not able to provide additional immunohistochemistry data for alpha 2u-globulin to confirm the composition of intracytoplasmic hyaline droplets in the kidney cells and validate the diagnosis of alpha 2u-globulin nephropathy in male rats. Despite the absence of these data and given that chronic progressive nephropathy (CPN) and alpha 2u-globulin nephropathy are specific to male rats (Frazier et al., 2012; Hard & Khan, 2016), both effects are considered by the Panel as not relevant to humans.

In the liver, periportal vacuoles, which are commonly observed in preclinical studies with rodents, occurred with higher frequency in the controls than in the treated groups and they were not considered toxicologically relevant. Diffuse hepatocyte cytoplasmic vacuoles were observed post administration of the test substance at all treatment doses in male rats at low-dose, mid-dose and high-dose, and in the high-dose female rats, but not in the controls (Maronpot, 2015). According to Maronpot (2015), 'the hepatocellular vacuolation was minimal to mild and uniform across dose groups without a dose-response in severity. This hepatocyte cytoplasmic vacuolation affected a minority of the hepatic lobules and there was no associated hepatocellular cytotoxicity accompanying the vacuolation.' According to Seely (2012), liver vacuoles of minimal severity were observed across all doses in males and in high-dose females, whereas vacuoles of mild severity were seen more in high-dose males compared to low- and mid-dose males.

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TABLE 14 Selected histopathology findings in Sprague Dawley rats, excluding early deaths. Statistically significant findings are indicated in bold.

	Males				Females			
Dose (mg/kg bw per day)	**0**	**100**	**300**	**1000**	**0**	**100**	**300**	**1000**
Animals examined (*N*)	11	12	11	12	12	12	12	12
Adrenal, cortical cytoplasmic vacuolation[a]				2				3
Stomach, minimal thickening of limiting ridge[a]	0	0	0	4	0	0	1	**6***
Jejunum, dilated lacteals[a]	0	0	0	**11****	0	0	0	**9****
Caecum, minimal cell infiltration in lamina propria[a]	1	1	4	5	1	0	0	2
Kidney, alpha 2u-globulin nephropathy[a]	0	**7****	**10****	**12****	0	0	0	0
Liver, minimal to moderate periportal hepatocyte fine vacuoles[a]	11	6	**3****	**4****	4	1	2	8
Liver, diffuse large hepatocyte cytoplasmic vacuoles[a]	0	**5***	**11****	**11****	0	0	2	**11***
Liver, centrilobular cytoplasmic vacuoles (minimal severity)[b]	0	**8**	**10**	**5**	0	0	3	**11**
Liver, centrilobular cytoplasmic vacuoles (mild severity)[b]	0	**2**	**2**	**7**	0	0	0	**1**

[a]As reported by Maronpot (2015).
[b]As reported by Seely (2012).
*$p \leq 0.05$. **$p \leq 0.01$.

The Panel notes a number of changes in the 90-day study (Maronpot, 2015; Oda, 2006; Seely, 2012) in a variety of parameters examined (haematological, serum biochemical and histopathological) in animals administered with the product as it is intended to be placed on the market. Platelet counts were reduced, whereas PT and APTT were increased in the 300 and/or 1000 mg/kg bw per day groups. Moreover, statistically significant changes were observed in some globulin isoforms in the 300 and 1000 mg/kg bw per day groups. Statistically significant increased incidence of diffuse liver cytoplasmic vacuoles was observed starting from the dose of 100 mg/kg bw per day in male animals.

The Panel notes that the presence of chalcone and coumarin compounds in the NF could have an impact on coagulation, which is in line with observations in the submitted 90-day study. Chalcones have been shown to inhibit thromboxane formation and exhibit antiplatelet activity (Ko et al., 2004; Lin et al., 2001; Lo et al., 2009). Xanthoangelol, one of the two major chalcones in ashitaba plant, has demonstrated antiplatelet activities in vivo (Ohkura et al., 2016). The NDA Panel has previously noted that coumarins can also exert an anticoagulant effect (EFSA NDA Panel, 2011). Chalcones have been reported to exhibit several biological activities that may be partly due to their structure containing a Michael acceptors motif (Jackson et al., 2017). Furthermore, data from the literature on chalcones show that they also have effects on lipids. Administration of *A. keiskei* extract for 6 weeks in a rat model of hyperlipidaemia and hypertension resulted in elevated serum levels of cholesterol and phospholipid, and decreased liver triglycerides (Ogawa et al., 2003).

The Panel considers that given the minimal severity observed regarding the diffuse hepatocyte cytoplasmic vacuoles along with absence of other hepatotoxic effects (i.e. liver weight, liver enzymes) in the 100 mg/kg bw per day group, and considering the effects in haematological parameters, the lowest dose (100 mg/kg bw per day) as the overall no observed adverse effect level (NOAEL).

The applicant provided another 90-day study (Kukulinski, 2013, unpublished report) performed in Sprague Dawley rats. The study was performed as a follow-up study with primary emphasis on hepatic effects and recovery/reversibility of these effects. Eight animals (4/sex) were administered for 90 consecutive days 600 mg/kg bw per day of the product as it is intended to be placed on the market. Four of these animals were kept for an additional 90-day recovery period. In parallel, a control group of four animals was administered with 1% aqueous gelatin and two out of them were kept for another 90-day post-treatment. According to the applicant, the study was performed 'in the spirit of GLP' and no OECD guidelines were followed. Hepatic changes observed in test males and females after 90 days of administration described as mild hepatocellular hypertrophy and minimal to mild hepatocellular vacuolation. The hypertrophy was most pronounced in cells in the centrilobular zone and surrounding the hepatic vein. The hypertrophy appeared to be greater in treated males compared to females. The vacuolar change was characterised as minimal to mild and evidenced by the presence of very small singular or multiple vacuoles mainly in periportal hepatocytes. The authors reported that hepatic changes observed in this non-GLP non OECD-study disappeared during the 90-day recovery period. The potential reversibility of the effects was considered out of scope by the Panel for this assessment, and only observations under continuous exposure were considered relevant.

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3.10.4 | Human data

Human studies using the product as it is intended to be placed on the market as a test substance were performed by the applicant, and are summarised in Table 15. These studies were not principally designed to assess the safety of the NF; however, safety-related observations were evaluated and are described below.

TABLE 15 Summary of human data.

Reference	Study design	Study population	Duration of study	Doses	Safety-related parameters investigated
Ohnishi and Hackel (2017)	Double-blind, randomised, placebo-controlled	26 overweight adults ($25 \le BMI < 30$ kg/m^2; age 40–65 years)	8 weeks	200 mg product as it is intended to be placed on the market versus placebo	Subjective symptoms, blood tests, urine test and physical exams (heart rate, blood pressure)
Tomita (2017) Confidential and proprietary	Double-blind, randomised, placebo-controlled	42 overweight adults ($25 < BMI < 30$ kg/m^2; age 20–64 years)	12 weeks	200 mg product as it is intended to be placed on the market VS placebo	Incidence of side effects and adverse events, physical examination except for efficacy endpoints, clinical examination, clinical chemistry (blood tests, urinalysis), and haematology
Hewlings et al. (2018)	Double-blind, randomised, placebo-controlled	60 adults with metabolic syndrome ($25 \le BMI \le 40$ kg/m^2; age 35–70 years)	12 weeks	220 mg product as it is intended to be placed on the market VS placebo	Laboratory analyses assessed metabolic markers, clinical chemistry, and haematology

Abbreviation: BMI, body mass index.

A double-blind, randomised, placebo-controlled study was designed to assess the effect of the product as it is intended to be placed on the market on body fat reduction in overweight male and female adults (Ohnishi & Hackel, 2017). In this study, 13 subjects received the product as it is intended to be placed on the market at a dose of 200 mg per day and 13 subjects a placebo, once per day (after dinner) for 56 consecutive days. The subjects were monitored for subjective symptoms, and blood tests, urine test and physical exams (heart rate, blood pressure) were performed to determine the safety of the test material. Subjects were tested at baseline, week 4 and week 8 (day 56). The authors reported no significant changes in any of the safety parameters measured.

In a randomised, placebo-controlled, double-blind, parallel-group study, the product as it is intended to be placed on the market was investigated in parallel to its potential to reduce body fat in humans (Tomita, 2017). In total, 42 subjects were distributed equally to a treatment and a control group receiving 200 mg per day of the product as it is intended to be placed on the market or a placebo, respectively, for 12 weeks. Physical examinations and sample collections (blood and urine) were performed at baseline, week 4, 8 and 12 for all subjects. The authors reported a statistically significant reduction in serum total protein at 4 weeks compared to the control group, which returned to comparable levels at week 12 as the only variation in safety parameters observed during this study.

The article from Hewlings et al. (2018) reports results from a randomised, double-blind, placebo-controlled study performed with 60 subjects (30 women and 30 men) exhibiting metabolic syndrome. The subjects were divided equally into the treatment and control group receiving 220 mg of the product as it is intended to be placed on the market or a placebo, respectively, once per day (with dinner) for 12 weeks. Laboratory analyses monitoring metabolic, clinical chemistry and haematology markers were performed at baseline, week 8 and 12. The authors reported a significant increase in triglycerides at week 12 in the treatment group relative to the placebo group ($p = 0.036$; effect size = 0.8), which was not accompanied by changes in other lipid levels (i.e. total cholesterol, HDL, LDL). Due to high variation in baseline values among the groups during the study design, this exploratory pilot study is of limited value for the safety assessment of the NF.

The Panel notes that the human studies provided by the applicant were primarily designed to investigate putative beneficial effects exclusively in overweight individuals or individuals with metabolic syndrome at intakes up to 220 mg per day and addressed only a limited number of safety-related endpoints such as physical and clinical examination (haematology, blood biochemistry and urinalysis).

The Panel concludes that the available human data, although with some limitations, do not raise safety concerns.

3.11 | Allergenicity

The NF, which is the sap from *Angelica keiskei* plant, is composed on average of 0.3% protein (below 0.45% based on specifications, Table 9). Ashitaba plant belongs to the Apiaceae family, in which several allergens have been identified, such as parsley, coriander, cumin, aniseed, bell pepper and pepper. Celery allergy is closely linked to birch and mugwort pollen

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sensitisation and it is often referred to as Birch-Mugwort-Celery-syndrome (Matricardi et al., 2016). Following an EFSA request, the applicant performed a literature search on the allergenicity, and cross-reactivity of ashitaba and other allergic foods such as celery. One case report was identified and related to the intake of the commercially available *Euglena*-containing product that also contained *A. keiskei*. However, the study authors concluded that the method used to test the allergenicity of *Euglena*-containing product including *A. keiskei* was unreliable (Utsunomiya et al., 2019). A study reported that bioactive constituents of *A. keiskei* including xanthoangelol B, C and E possessed anti-allergic activity, because they inhibited histamine release from rat mast cells. In the same study, it was also referred that xanthoangelol and 4-hydroxyderricin had the opposite effect enhancing histamine release (Kil et al., 2017). No other studies were identified on the potential of cross-reactivity of *A. keiskei* with other allergenic foods.

The applicant analysed the presence of celery (*Apium graveolens*) allergens in the product as it is intended to be placed on the market performing qualitative polymerase chain reaction (PCR) analyses using primers for the detection of mannitol dehydrogenase (*Mtd)* gene. The applicant noted the absence of *Mtd* gene in the four tested batches of the product as it is intended to be placed on the market. Furthermore, the applicant provided analytical data on allergens listed in Annex II of the Regulation (EU) No 1169/2011[7] in the product as it is intended to be placed on the market for three batches and found none of the allergens. No tests assessing the cross-reactivity of the NF with other allergic foods were carried out.

Human studies using the product as it is intended to be placed on the market showed no allergic reactions (Hewlings et al., 2018; Maronpot, 2015; Ohnishi & Hackel, 2017; Tomita, 2017).

The amount of protein contribution from ashitaba sap would be low (i.e. approximately up to 2.34 mg per day or 0.033 mg/kg bw per day for a 70 kg adult) at the proposed use level (780 mg per day). Therefore, considering the low amounts of proteins in the NF and the proposed use level, the Panel considers that the risk of allergenicity is low.

The Panel concludes that the risk of the NF to trigger allergic reactions in the target population under the proposed conditions of use is unknown but expected to be low.

4 | DISCUSSION

The NF which is the subject of the application is ashitaba sap, which is collected from harvested stems of ashitaba plants (*A. keiskei*) originated from certified organic farming. The principal constituents are chalcones (1%–2.25%) and furanocoumarins (< 0.01%).

The applicant intends to market the NF in the form of a freeze-dried powder, containing around 30% of ashitaba sap powder and 70% cyclodextrins, standardised for the chalcone content (at least 8%) to be used in food supplements at maximum dose of 200 mg per day, corresponding to 780 mg of the NF. The target population is adults, excluding pregnant and lactating women.

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

Taking into account the test results provided and considering the nature, source and production process of the NF, the Panel considers that there are no concerns regarding genotoxicity.

In the 90-day oral toxicity study performed by the applicant with the product as intended to be placed on the market, changes in coagulation indices and platelet counts were observed. Moreover, increased serum cholesterol, triglycerides and phospholipid as well as jejunal dilated lacteals and diffuse liver cytoplasmic vacuolar changes were noted indicating lipid metabolism effects. The Panel considers that the overall NOAEL of the provided 90-day oral toxicity study (Maronpot, 2015; Oda, 2006; Seely, 2012) is the lowest dose tested, i.e. 100 mg/kg bw per day.

Based on the identified NOAEL of 100 mg/kg bw per day and by applying an uncertainty factor of 200 (10 (interspecies variability) × 10 (intraspecies variability) × 2 (subchronic to chronic study duration)), the Panel establishes a safe level of 0.5 mg/kg bw per day for the product as it is intended to be placed on the market. For the intended target population, i.e. adults, with a default body weight of 70 kg, this corresponds to an intake of 35 mg per day of the product as it is intended to be placed on the market. Based on the ratio of the NF (ashitaba sap, containing ~92.9% of water) to the product as it is intended to placed on the marker (30% ashitaba sap powder and 70% cyclodextrins) as presented in Section 3.7.2, the corresponding intake of the NF is 137 mg per day, which is lower than the use level proposed by the applicant.

5 | CONCLUSIONS

The Panel concludes that the NF, ashitaba sap from the stems of *Angelica keiskei* plants, is safe for the target population at intake levels up to 137 mg per day, corresponding to 35 mg per day of the product as intended to be placed on the market (30% ashitaba sap powder and 70% cyclodextrins).

[7]Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004 Text with EEA relevance. OJ L 304, 22.11.2011, p. 18–63.

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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 data claimed as proprietary by the applicant: data on the characterisation of botanical source, certificates of raw materials, methods of analysis, certificates of analysis, two bacterial revers mutation tests (Krul, 2002, unpublished; Joshi, 2023a, unpublished), an in vitro mammalian chromosomal aberration test (de Vogel, 2003, unpublished), an in vitro mammalian cell micronucleus test (Joshi, 2023b, unpublished), an in vivo micronucleus test (Cardoso & Licollari, 2014, unpublished), an acute oral toxicity study (Prinsen, 2002, unpublished), two 90-day oral toxicity studies (Oda, 2006, unpublished; Kukulinski, 2013, unpublished), a report on histopathological findings observed in a 90-day oral toxicity study (Seely, 2012, unpublished), a human study (Tomita, 2017, unpublished).

6 | STEPS TAKEN BY EFSA

1. On 19/12/2019 EFSA received a letter from the European Commission with the request for a scientific opinion on the safety of ashitaba sap powder. Ref.Ares(2019)7829847.
2. On 19/12/2019, a valid application on ashitaba sap powder, which was submitted by Japan Bio Science Laboratory (JBSL)-USA, Inc, was made available to EFSA by the European Commission through the Commission e-submission portal (NF 2019/1247) and the scientific evaluation procedure was initiated.
3. On 08/04/2020, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
4. On 17/01/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
7. On 15/02/2022, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
8. On 12/04/2022, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
9. On 27/04/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
10. On 23/05/2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
11. On 25/05/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
12. On 23/08/2323, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
13. On 12/09/2023, EFSA requested the applicant to provide additional information to accompany the application and the scientific evaluation was suspended.
14. On 15/10/2023, additional information was provided by the applicant through the Commission e-submission portal and the scientific evaluation was restarted.
15. During its meeting on 01/02/2024, the NDA Panel, having evaluated the data, adopted a scientific opinion on the safety of ashitaba sap as a NF pursuant to Regulation (EU) 2015/2283.

ABBREVIATIONS

ADME	absorption, distribution, metabolism and excretion
ALP	alkaline phosphatase
AOAC	Association of Official Analytical Chemists
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
BAM	bacteriological analytical manual
BMI	body mass index
BUN	blood urea nitrogen
bw	body weight
CBPI	cytokinesis block proliferation index
CFU	Colony Forming Units
CHO	Chinese Hamster Ovary cells
CPN	chronic progressive nephropathy
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
EA	ethyl acetate
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice

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H&E	haematoxylin and eosin
HDL	high-density lipoprotein
HPLC	high-performance liquid chromatography
HPLC-UV	high-performance liquid chromatography- ultra violet detection
HPTLC	high performance thin layer chromatography
ICP-MS	inductively-coupled plasma mass spectrometry
ICR	Institute of Cancer Research
ISO	International Organisation for Standardisation
JBSL	Japan Bio Science Laboratory
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LDL	low-density lipoprotein
LOQ	limit of quantification
Mtd	mannitol dehydrogenase
NCE	normochromatic erythrocytes
NDA	Nutrition, Novel Foods and Food Allergens
NF	Novel Food
NOAEL	no observed adverse effect level
OECD	Organization for Economic Cooperation and Development
PAH	polycyclic aromatic hydrocarbons
PCEs	polychromatic erythrocytes
PCR	polymerase chain reaction
PT	prothrombin time
SD	standard deviation
SPF	specific pathogen free
TG	test guidelines
USDA	United States Department of Agriculture
U.S. FDA	United States Food and Drug Administration
v/v	volume per volume
w/v	weight per volume

CONFLICT OF INTEREST

If you wish to access the declaration of interests of any expert contributing to an EFSA scientific assessment, please contact interestmanagement@efsa.europa.eu.

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PANEL MEMBERS

Dominique Turck, Torsten Bohn, Jacqueline Castenmiller, Stefaan De Henauw, Karen Ildico Hirsch-Ernst, Alexandre Maciuk, Inge Mangelsdorf, Harry J McArdle, Androniki Naska, Kristina Pentieva, Alfonso Siani, Frank Thies, Sophia Tsabouri, and Marco Vinceti.

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