COMISIÓN DEL CODEX ALIMENTARIUS **S**

Organización de las Naciones Unidas para la Alimentación y la Agricultura

Organización Mundial de la Salud

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Tema 2 del programa **CX/CF 21/14/2-Add.1**

Marzo de 2021

PROGRAMA CONJUNTO FAO/OMS SOBRE NORMAS ALIMENTARIAS

COMITÉ DEL CODEX SOBRE CONTAMINANTES DE LOS ALIMENTOS

Décima cuarta reunión
(virtual)
3-7 y 13 de mayo de 2021

ASUNTOS REMITIDOS AL COMITÉ POR EL COMITÉ COORDINADOR FAO/OMS PARA AMÉRICA DEL NORTE Y EL PACÍFICO SUDOCCIDENTAL: SOLICITUD DE UNA EVALUACIÓN DE LA INOCUIDAD DE LA ESCOPOLETINA EN EL ZUMO (JUGO) FERMENTADO DE FRUTO DE NONI

(Preparado por la Secretaría del Codex)

Antecedentes

1. En la novena reunión (2006) del Comité Coordinador FAO/OMS para América del Norte y el Pacífico Sudoccidental (CCNASWP), el Coordinador Regional, Samoa, informó al Comité Coordinador acerca de su deseo de comenzar los trabajos sobre la elaboración de una norma para los productos a base de noni. El CCNASWP, en dicha reunión, convino en solicitar a un Grupo de trabajo por medios electrónicos (GTe) que preparara un documento de debate sobre la necesidad de elaborar dicha norma.[1]

2. En la décima reunión (2008) del CCNASWP, se presentó un documento de debate, que había sido preparado por un GTe dirigido por el Coordinador Regional, Tonga, y se convino en que era necesario llevar a cabo consultas más amplias con los países productores y la industria antes de empezar a redactar un proyecto de norma para los productos a base de noni.[2]

3. En la 11.ª reunión (2010) del CCNASWP, se presentó una propuesta de nuevo trabajo sobre la elaboración de una norma para los productos a base de noni, preparada por Tonga con la ayuda de otros miembros[3]. El Comité Coordinador, entre otras cosas, señaló la necesidad de aclarar mejor si era preciso llevar a cabo estudios adicionales sobre la inocuidad de los productos a base de noni. Asimismo, el Comité Coordinador convino en establecer un GTe para que revisara el documento de debate con miras a su examen en la siguiente reunión.

4. En la 12.ª reunión (2012)[4] del CCNASWP, Tonga presentó un documento de debate revisado[5], el cual incluía como anexo un documento de proyecto, y explicó que la inocuidad de los productos a base de noni se basaba en la dilatada historia de utilización en el Pacífico, y que la norma abarcaría tres productos principales, a saber: i) el puré de fruto de noni; ii) los zumos (jugos) fermentados de fruto de noni, y iii) las hojas secas de noni. Se señaló que la Autoridad Europea de Seguridad Alimentaria (AESA) había aprobado algunos productos a base de noni como "productos alimenticios nuevos", y que en el documento de proyecto se proporcionaba información sobre la inocuidad de los productos a base de noni, como la evaluación sobre inocuidad de 2006 de la AESA. En respuesta a las peticiones de asistencia científica, la Representante de la OMS aclaró que, en vista de los recursos limitados, era necesario establecer un orden de prioridades y recordó a los países que la elaboración de los documentos de proyecto, especialmente la evaluación sobre la inocuidad, era cometido de los países. El CCNASWP, en dicha reunión, convino en restringir el ámbito de aplicación de la norma a los zumos (jugos) fermentados de fruto de noni, que se producían y comercializaban principalmente en los países insulares del Pacífico, y apuntó que una vez se hubiera establecido la norma, el Comité Coordinador podría pensar en la posibilidad de ampliarla a otros tipos de productos a base de noni. El Comité se mostró de acuerdo en iniciar la nueva tarea de elaborar una norma regional para los zumos (jugos) fermentados de noni, y en que Tonga, con la ayuda de Australia, revisara el documento de proyecto del nuevo trabajo con miras a presentarlo en la 68.ª reunión del Comité Ejecutivo (2013). El CCNASWP acordó establecer un GTe, presidido por Tonga, para que preparara un anteproyecto de norma regional para los zumos (jugos) fermentados de fruto de noni con miras a su examen por el CCNASWP en su 13.ª reunión.

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1 ALINORM 07/30/32, párr. 84
2 ALINORM 09/32/32, párr. 47
3 REP11/NASWP, párrs. 101-103
4 REP13/NASWP, párrs. 126, 131, 133-136
5 CX/NASWP 12/12/9

5. La Comisión del Codex Alimentarius (CAC), en su 36.º período de sesiones (2013), aprobó el nuevo trabajo sobre la elaboración de una norma regional para los zumos (jugos) fermentados de fruto de noni[6] como había recomendado el Comité Ejecutivo en su 68.ª reunión[7] basándose en el documento de proyecto[8].

6. En la 13.ª reunión (2014)[9] del CCNASWP, se presentó un anteproyecto de norma regional para los zumos (jugos) fermentados de fruto de noni[10]. El Comité Coordinador examinó las solicitudes de asesoramiento científico señaladas en el documento de proyecto[8], así como una petición para la determinación de niveles inocuos de ingesta para la escopoletina, y se recordó que las solicitudes de asesoramiento científico tenían que estar claramente definidas para que la FAO y la OMS pudieran dar una respuesta apropiada. En dicha reunión, el CCNASWP acordó solicitar el asesoramiento del Comité del Codex sobre Contaminantes de los Alimentos (CCCF) acerca de un nivel máximo inocuo para la escopoletina, junto con un método de análisis. En ese momento no era necesario ningún otro asesoramiento científico. Una delegación manifestó la opinión de que una vez recibida esta información podía quedar resuelta la cuestión relativa a la ingesta diaria máxima recomendada de zumo (jugo) fermentado.

7. El CCCF, en su novena reunión[11] (2015), acordó incluir la escopoletina en la Lista de prioridades de contaminantes y sustancias tóxicas presentes naturalmente para su evaluación por el Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios (JECFA) (una evaluación completa de la evaluación toxicológica y la evaluación de la exposición de los zumos (jugos) fermentados de fruto de noni).

8. En su décima reunión[12] (2016), el CCCF fue informado de que la escopoletina seguía formando parte de la Lista de prioridades para evaluación por el JECFA, y de que se consultaría al CCNASWP sobre el estado de la norma para el zumo (jugo) de noni y la disponibilidad de datos.

9. En la 14.ª reunión (2016)[13] del CCNASWP, Tonga presentó una revisión del anteproyecto de norma regional para los zumos (jugos) fermentados de fruto de noni[14] y la Secretaría del Codex recordó la petición que el CCCF, en sus reuniones novena y décima, había realizado sobre el estado de la norma y la disponibilidad de datos para que el JECFA pudiera llevar a cabo una evaluación completa de riesgos con respecto a la escopoletina. El Representante de la FAO aclaró que los datos solicitados por el JECFA para llevar a cabo una evaluación de la inocuidad de la escopoletina incluían todos los datos disponibles sobre: i) toxicidad de la escopoletina; ii) presencia de escopoletina en el zumo (jugo) de noni y otros productos básicos en los que la escopoletina podía estar presente, iii) datos sobre el consumo de zumo (jugo) fermentado de fruto de noni y otros productos que podrían contener escopoletina. La Secretaría instó además a los miembros del Comité Coordinador a que proporcionaran datos para la evaluación del JECFA sobre la inocuidad de la escopoletina y siguió alentando a los miembros a que proporcionasen información al CCCF y una justificación sólida a fin de garantizar que se concede máxima prioridad a esta sustancia para la evaluación del FECFA. En dicha reunión, el CCNASWP se mostró de acuerdo en volver a convocar el GTe, dirigido por Tonga, para redactar de nuevo el anteproyecto de norma, acordó informar al CCCF del estado de elaboración de la norma e instó a los miembros del CCNASWP a que proporcionaran al JECFA datos sobre la inocuidad y toxicidad de la escopoletina (toxicidad, presencia y datos sobre el consumo).

10. El CCCF, en sus reuniones 11.ª[15] (2017), 12.ª[16] (2018) y 13.ª[17] (2019), tomó nota de que la escopoletina seguía formando parte de la Lista de prioridades para evaluación por el JECFA y de que el CCNASWP seguía trabajando en una norma para los zumos (jugos) de fruto de noni y la disponibilidad de datos. En su 11.ª reunión, el CCCF fue informado de que con base en un examen rápido de la bibliografía realizado por los Estados Unidos de América no había datos suficientes para obtener un valor de referencia basado en la salud. El CCCF, en su 13.ª reunión, acordó solicitar al CCNASWP si a) deseaban mantener la escopoletina en la Lista de prioridades y, en caso afirmativo, b) cuándo habría datos adecuados al respecto[17].

11. El CCNASWP, en su 15.ª reunión[18] (2019), en respuesta a la solicitud del CCCF en su 13.ª reunión, confirmó que

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6 REP13/CAC, Apéndice VI
7 REP13/EXEC, párr. 65
8 CX/EXEC 13/68/4
9 REP15/NASWP, párrs. 34, 35, 51, 52
10 CX/NASWP 14/13/6
11 REP15/CF, párr. 147
12 REP16/CF, párr. 163, Apéndice VI
13 REP17/NASWP, párrs. 61-68
14 CX/NASWP 16/14/9
15 REP17/CF, Apéndice XII
16 REP18/CF, Apéndice X
17 REP19/CF, párr. 168, Apéndice X
18 REP20/NASWP, párr. 74

deseaba mantener la escopoletina en la Lista de prioridades del JECFA y pidió a los países miembros que generasen y enviasen datos adecuados para la evaluación, que aborda tanto la toxicidad como la exposición. El Comité Coordinador pidió también a la FAO y la OMS que organizasen una nueva solicitud de datos para la evaluación de la inocuidad de la escopoletina. El Representante de la FAO recordó que se requería un conjunto completo de datos que incluyera la exposición y la toxicidad.

Examen de la disponibilidad de datos

12. Después de la 15.ª reunión del CCNASWP, los países de la región han intensificado sus esfuerzos para obtener los datos solicitados, pero esta labor se ha visto limitada a consecuencia de numerosos factores, entre ellos la pandemia de la enfermedad por coronavirus (COVID-19), y, hasta el momento, no se han presentado nuevos datos.

13. La Secretaría del Codex, reconociendo la falta de datos toxicológicos suficientes, hacia finales de 2019 encargó a un consultor que evaluara la información toxicocinética, biológica, bioquímica y toxicológica disponible sobre la escopoletina en la bibliografía científica publicada, e identificara las lagunas de datos con objeto de sustentar la solicitud de asesoramiento del Comité Coordinador al CCCF sobre un nivel máximo inocuo para la escopoletina en los zumos (jugos) de fruto de noni y contribuir así a la labor en curso del Comité Coordinador en la elaboración de una norma regional para los zumos (jugos) fermentados de fruto de noni.

14. En febrero de 2020, se envió a la Secretaría del Codex un examen que abarca parte del trabajo preliminar necesario para facilitar una futura evaluación del JECFA (Anexo I).

15. El examen mostró que:

    i. Se necesitarían datos más detallados sobre la concentración de escopoletina en los zumos (jugos) de fruto de noni en comercio para facilitar el asesoramiento adicional.

    ii. Los datos toxicocenéticos disponibles derivados de estudios en animales y seres humanos eran suficientes, fiables y coherentes, y que los numerosos datos biológicos y bioquímicos mostraban que la escopoletina posee numerosas actividades *in vitro,* algunas de las cuales han sido demostradas también *in vivo*.

    iii. Los datos disponibles sobre toxicidad son muy escasos. No hay el tipo de estudios convencionales sobre la toxicidad que suele ser necesario para llevar a cabo una evaluación de riesgos.

16. El examen identificó lagunas de datos importantes, como la falta de datos sobre la toxicidad de las dosis repetidas, la genotoxicidad y la toxicidad en la reproducción y el desarrollo. Asimismo, es preciso confirmar que no hay neurotoxicidad. Se recomienda que se lleven a cabo estudios toxicológicos que abarquen estas esferas clave como mínimo necesario para poder realizar una evaluación de riesgos y sustentar la propuesta de un límite máximo para la escopoletina en los zumos (jugos) de fruto de noni.

17. Con base en dicho examen, se recomienda que se lleven a cabo nuevos estudios toxicológicos que comprendan un estudio de la toxicidad de las dosis repetidas en ratas, una serie de pruebas de genotoxicidad *in vitro*, una prueba de la toxicidad en la reproducción en ratas, y un estudio de la toxicidad en el desarrollo en ratas, a poder ser de conformidad con las directrices pertinentes para la realización de ensayos de la Organización para la Cooperación y el Desarrollo Económicos (OCDE) y las directrices internacionales para las buenas prácticas de laboratorio (BPL).

Próximos pasos

18. La Secretaría presentará el mencionado informe en la próxima reunión del Comité Coordinador para su examen en el contexto del trabajo sobre la norma para los zumos (jugos) de fruto de noni, y la adopción de una decisión sobre ulteriores medidas.

Recomendaciones

19. Se recomienda que este documento se examine junto con el documento CX/CF 21/14/18 en el marco del tema 20 del programa (evaluaciones del JECFA, Lista de prioridades).

Se invita al CCCF a:

20. Tomar nota de las conclusiones del examen toxicológico que figura en el Anexo del presente documento.

21. Mantener la escopoletina en la Lista de prioridades en espera de la respuesta del Comité Coordinador y el asesoramiento al CCCF sobre si, con base en las conclusiones del examen toxicológico, los países de la región del Pacífico sudoccidental podrían proporcionar los datos y los estudios necesarios para sustentar la evaluación del JECFA sobre la escopoletina y su posterior examen por el CCCF.

22. Alentar a otros miembros del Codex y observadores interesados en los productos a base de fruto de noni/escopoletina, además de los países de la región del Pacífico sudoccidental, a proporcionar datos o información pertinentes a la FAO/OMS, o generarlos con ese fin, que posibiliten que el JECFA lleve a cabo la evaluación de este compuesto y su posterior consideración por el CCCF.

ANEXO
SOLO EN IDIOMA ORIGINAL

SCOPOLOTIN REPORT:
REVIEW OF AVAILABLE TOXICITY DATA, IDENTIFICATION OF DATA GAPS, ADVICE ON ADDRESSING DATA GAPS
(For information)

Report prepared for: Food and Agricultural Organization of the United Nations, Codex Secretariat, Rome, Italy

Date: 15 February 2020

Author: Susan M Barlow BSc PhD DiRCPath Brighton, UK

EXECUTIVE SUMMARY

Aim of the review

The aim of this review was to evaluate the toxicokinetic, biological, biochemical and toxicological information available on **scopoletin** in the open scientific literature and to identify data gaps, in order to support the request by the Coordinating Committee for North America and South West Pacific (CCNASWP) to the Codex Committee on Contaminants in Food (CCCF) for advice on a safe maximum level for scopoletin in Noni juice and to support the ongoing work of the CCNASWP in developing a Draft Regional Standard for Fermented Noni Juice. The FAO/WHO Joint Expert Committee on Food Additives (JECFA) has been asked to evaluate scopoletin. This review covers some of the groundwork required to facilitate an eventual JECFA evaluation.

Occurrence of scopoletin in Noni juice

Scopoletin (6-methoxy-7-hydroxycoumarin) is a naturally occurring analogue of coumarin and is known as one of the simple coumarins. It is a constituent of Noni fruit. Concentrations of scopoletin in Noni juice reported in the open scientific literature range from 100-235 µg/mL, depending, in part, on the ripeness of the fruit and whether or not it is fermented. More extensive data on concentrations of scopoletin in Noni juice in commerce would also be required to facilitate further advice. A data call for this information has already been issued by CCCF.

Toxicokinetics

The available toxicokinetic data from animal and human studies are sufficient, reliable and consistent. They show that scopoletin, whether given as such, or as a component of noni or other traditional herbal medicines, is absorbed very rapidly from the gastrointestinal tract, reaching peak blood levels within 20 minutes or less, and that absorption is dose-dependent. Elimination, principally in the urine, is also rapid. Urinary metabolites are scopoletin glucuronide and scopoletin sulfate. Overall, it has low oral bioavailability (1-7%).

Biological and biochemical data

Review of the extensive biological and biochemical data available shows that scopoletin has numerous activities in vitro, some of which have also been demonstrated in vivo. These activities include antioxidant, anti-inflammatory, antiproliferative, antihypertensive, and antidiabetic activities. It ameliorates lipid disturbances in animal models of liver toxicity. In vitro and in neural tissue it inhibits acetylcholinesterase activity. In vivo, there is limited evidence that it affects serotonergic, noradrenergic and dopaminergic activity in the central nervous system in a manner that could be interpreted as neuroprotective. Mechanistic data, mostly from in vitro studies, but sometimes supported by in vivo studies, show that scopoletin can influence several cellular signalling pathways that control, *inter alia*, free radical scavenging, apoptosis, angiogenesis, inflammation, lipid and glucose metabolism, and intracellular calcium mobilisation.

Toxicological data

In contrast, the available data on toxicity are very sparse. There are no conventional toxicity studies of the type that are usually necessary for risk assessment.

After review of the toxicokinetics, and the biological, biochemical and toxicological data that were available, significant data gaps were identified, including absence of repeated-dose, genotoxicity, reproductive, and developmental toxicity data. Confirmation of the absence of neurotoxicity is also needed. Toxicological studies covering these key areas are recommended as the minimum necessary to allow risk assessment and support a proposal for an upper limit for scopoletin in Noni juice.

Recommendations for toxicological studies needed

1. A repeated-dose toxicity study in the rat, minimum duration of 90-days, to include measurement of brain, red blood cell and plasma acetylcholinesterase at termination.
2. A battery of *in vitro* genotoxicity tests to cover the endpoints of mutagenicity, clastogenicity and aneugenicity. A bacterial reverse mutation assay in *Salmonella typhimurium* (5 tester strains, with and without metabolic activation) and an *in vitro* micronucleus assay would suffice to cover these three endpoints. In case of positive results *in vitro*, an appropriate *in vivo* study, to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo*, would be necessary.
3. A test for reproductive toxicity in the rat, such as a multigeneration study or an extended one-generation reproduction study would be necessary.
4. A developmental toxicity study in the rat would be necessary. If negative, then a study in the rabbit might also be necessary.

All the above studies should, ideally, be conducted in accordance with the relevant OECD test guidelines, which are available for all the proposed studies, and in accordance with international guidelines for Good Laboratory Practice (GLP).

1. REVIEW OF BIOLOGICAL AND TOXICITY DATA ON SCOPOLETIN

1.1 Background

Scopoletin (6-methoxy-7-hydroxycoumarin) is a naturally occurring simple analogue of coumarin. Coumarins belong to the benzopyrone chemical family. Coumarins are widely distributed in the plant world, in seeds, fruits, flowers, roots, leaves and stems, with the largest concentrations generally found in fruits and flowers (Matos et al., 2015).

Umbelliferone, esculatin and scopoletin are the most widespread coumarins in nature. Scopoletin is found in many edible plant families and their fruits, including noni, *Morinda citrifolia* L. (Matos et al., 2015). Noni is a member of the Rubiaceae plant family, which is widely found in tropical and subtropical regions (Yan et al., 2018). Scopoletin has been found in authentic noni fruit samples from all tropical regions around the world and has been identified in commercial noni juice products (West & Deng, 2010a, Deng et al., 2010b). In noni fruit, scopoletin occurs mainly as a glycoside, but during the fermentation used to produce some forms of noni juice, the glycosidic link is hydrolysed to produce the aglycone and scopoletin occurs in the free form (Westendorf, 2016). Ripe fruits contain much higher levels of scopoletin than unripe fruits and during the first 8 weeks of fermentation of ripe fruits, scopoletin concentrations increase (Yang SC et al., 2007). Traditional noni juice production is by drip-extraction and fermentation. Non-traditional methods use pressing to extract the juice and do not involve fermentation.

Plant extracts containing coumarins and some individual coumarins have a long history of use in traditional medicine due to their array of pharmacological and biological activities, with claims of preventive properties and beneficial treatment of diseases (Gnonlonfin et al, 2012; Venugopala et al., 2013; Matos et al., 2015). Concerning either noni juice or scopoletin, robust clinical data are lacking and there is little evidence of clinical efficacy in prevention or disease in humans (Potterat & Hamburger, 2007).

1.2 Aim of the review

The aim of this present review is to evaluate the biochemical, biological and toxicological information on scopoletin available in the scientific literature. This is in order to support the request by the Coordinating Committee for North America and South West Pacific (CCNASWP) to the Codex Committee on Contaminants in Food (CCCF) for advice on a safe maximum level for scopoletin and to support the ongoing work of the CCNASWP in developing a Draft Regional Standard for Fermented Noni Juice (CCNASWP, 2014, 2019). Accordingly, the FAO/WHO Joint Expert Committee on Food Additives (JECFA) has been asked to evaluate scopoletin. Occurrence data have been requested (FAO/WHO, 2017) and a full evaluation (toxicological assessment and exposure assessment) has been requested (FAO/WHO, 2018).

1.3 Literature searches

A comprehensive literature search was performed using the PubMed and PubChem databases of the US National Library of Medicine and the Registry of Toxic Effects of Chemical Substances (RTECS). Searches were made without date restriction unless otherwise stated, using the terms ‘scopoletin toxicity’ (79 hits), ‘scopoletin bioassay’ (54 hits), ‘scopoletin genotoxicity’ (2 hits), ‘scopoletin cancer’ (130 hits), ‘scopoletin pathology’ (53 hits), ‘scopoletin effects’ (2016-2019 only, 144 hits), and ‘coumarins genotoxicity’ (298 hits). Google searches were conducted using the search terms ‘scopoletin occurrence’, ‘scopoletin noni juice’ and ‘noni juice production’. Of the references retrieved and scanned, a total of 164 were relevant for the review after study of the full papers or abstracts.

1.4 Chemistry

The structure of scopoletin is shown below.

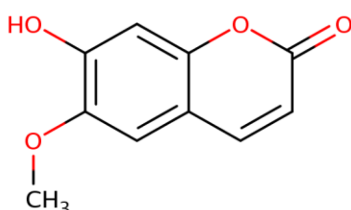

Figure 1. Chemical structure of scopoletin.

IUPAC Name: 7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one.
Molecular formula: $C_{10}H_8O_4$
Molecular mass: 192 Da (g/mol).
CAS Registry Number: 92-61-5.

1.5 Occurrence levels of scopoletin in Noni juice

Occurrence levels of scopoletin in Noni juice have been reported. The available data indicate that chemical compositions of Noni juice from different origins can be significantly different (Yan et al., 2018).

HPLC analysis of Noni juice obtained from fruits from Taiwan yielded concentrations in the range of 190 - 230 µg/mL in progressively fermenting juice from ripe fruits, compared with a range of 120 - 140 µg/mL in juice from unripe fruits (Yang et al., 2007).

West and Deng (2010), using thin layer chromatography (TLC) confirmed by HPLC, reported concentrations of scopoletin in the range 3.7 - 21.2 µg/mL in four commercial Noni fruit juice products originating from Tahiti, Dominican Republic, Hawaii, and Costa Rica, produced by different manufacturers, purchased at local markets or via the internet.

Yan et al. (2018), using a validated high-performance liquid chromatography (HPLC) with electrospray ionization triple quadrupole mass spectrometry (HPLC-ESI-MS/MS) method, reported concentrations ranging from 99 - 171 µg/mL in 6 commercially available samples of Noni juice purchased through the internet in 2016, originating from Wanzhou, Taiwan and Fijian (two samples per region), with markedly lower concentrations of around 2 µg/mL in two samples from Xisha.

Westendorf (2016) reported that the average concentration of scopoletin in freshly squeezed Noni juice was 114 µg/mL, whereas in 16 commercial samples it ranged from 9 - 235 µg/mL. Some of the differences were attributed to variations in the extent of rehydration of concentrate.

1.6 Toxicokinetics

1.6.1 Animals

1.6.1.1 Studies using pure scopoletin

Zeng et al. (2015) developed a more selective and more sensitive LC-MS/MS method for determining concentrations of scopolamine in rat plasma, which was validated. Scopoletin (98.0% purity) was obtained from a commercial supplier. It showed a good linear relationship over the range of 5 – 1000 ng/mL. The lower limit of quantification (LLOQ) for scopoletin in rat plasma was 5 ng/mL. The limit of detection (LOD) was 1 ng/mL at a signal-to-noise ratio of 3:1. Accuracy, precision, recovery, stability and matrix effect in rat plasma were within acceptable limits of variation. Male rats were given either 5 mg/kg bw intravenously or oral doses of 5, 10 or 20 mg/kg bw, with 5 rats per dose group. Rats were fasted for 12 h with free access to water prior to dosing. Blood samples were collected at 5, 10, 20, 30, 45, 60, 90, 120 min after scopoletin administration. There was no significant difference in mean residence time, $t_{1/2}$, T_{max} and clearance between the three orally-dosed groups. Oral doses were rapidly absorbed, reaching peak concentrations (T_{max}) at around 20 min, as shown in Figure 2. The $t_{1/2}$ after oral administration was 0.9, 0.7 and 0.5 h in 5, 10 and 20 mg/kg bw groups, respectively, and 0.8 h after intravenous administration, indicating that scopoletin was eliminated quickly *in vivo*. The oral bioavailability was similar for all 3 oral dose groups, ranging from 5.6 – 6.6%.

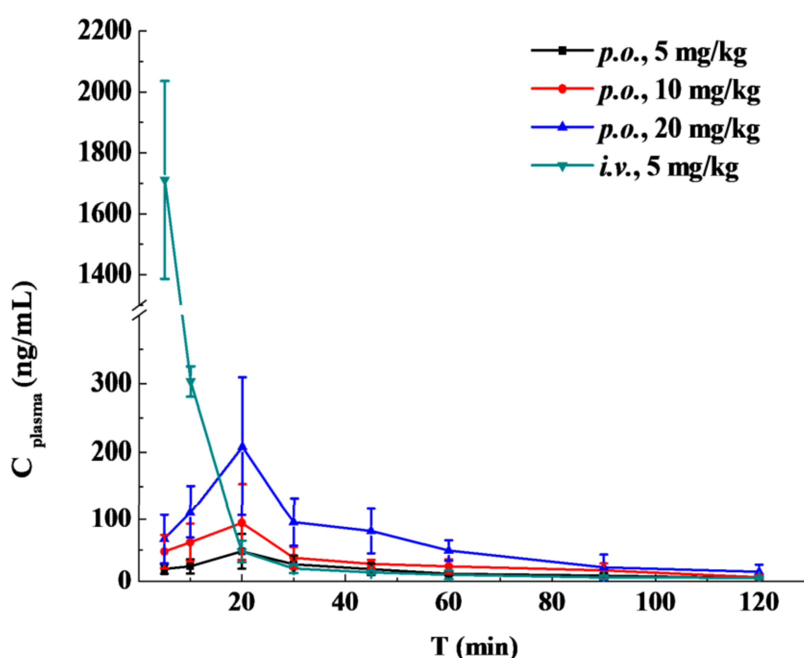

Figure 2. Mean scopoletin plasma concentrations over time after oral (*p.o.*, 5, 10 and 20 mg/kg) or intravenous (*i.v.*, 5 mg/kg) administration in rats (*n* = 5) (Zeng et al., 2015).

Zeng et al. (2017) compared the absorption of free scopoletin with that of scopoletin encapsulated into Solupus micelles. Scopoletin concentrations were determined using a high performance liquid chromatography (HPLC) system equipped with an ultraviolet detector. Groups of 5 male rats were fasted for 12 h then given a single oral dose of free scopoletin, scopoletin plus Soluplus in a physical mixture, or scopoletin in micelles. The scopoletin dose in each preparation was 100 mg/kg bw. Blood samples were taken at intervals of 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after dosing. Only the results from rats given free scopoletin are described. The kinetic profile of the free scopoletin was very similar to that of the 20 mg/kg dose in their earlier study (Zeng et al. 2015), with rapid absorption and very similar values for $t_{1/2}$ and T_{max}. Scopoletin concentrations were high in the duodenum and jejunum at 15 and 30 minutes, then declined; they increased in the ileum and colon at 60 and 120 minutes but not to the levels seen in duodenum and jejunum, indicating most was absorbed in the proximal intestine. The $t_{1/2}$ and T_{max} were 0.5 h and 0.3 h, respectively. C_{max} was 1741 µg/L. Tissue distribution measurements showed that scopoletin was distributed mainly in the liver, spleen, lung and kidney. The highest levels in liver and kidney were seen at 15 minutes.

Li B et al. (2019) used LC-MS/MS to measure scopolin and its metabolite, scopoletin, in plasma and urine after oral administration of scopolin to rats. Scopolin is a glycoside of scopoletin and would be expected to undergo rapid hydrolysis to scopoletin in the acidic conditions of the stomach. The LLOQ of scopoletin in rat plasma and urine was 5 ng/mL. Precision, accuracy, recovery, stability and matrix effect were within acceptable limits of variation. Rats were fasted for 12 h with free access to water, then given either 30 mg/kg bw scopolin by oral gavage. There were 6 male and 6 female rats per group. Blood samples were collected before dosing and at frequent intervals up to 300 min after dosing. Urinary excretion of scopolin and scopoletin was measured in 6 other male and female rats given 30 mg/kg bw orally and urine collected at intervals up to 72 h after dosing. Scopolin appeared very rapidly in blood with a T_{max} of 17 min in males and 20 min in females. The absolute bioavailability of scopolin was very low (around 1%). In those dosed orally, T_{max} for the appearance of scopoletin in plasma after dosing with scopolin was 14 min for males and 7 min for females. Plasma levels of scopoletin were less than 10% of C_{max} by 1 h after dosing and had reduced to very low values by 2 h after dosing. Scopoletin was very rapidly cleared from the plasma and was almost all excreted in urine within 4 h after dosing. Total elimination in urine took slightly longer in females than males.

Zhao et al. (2019) used a highly sensitive and selective method based on ultra-high-performance liquid chromatography combined with linear ion trap-Orbitrap tandem mass spectrometry (UHPLC-LTQ-Orbitrap-MS). The method was validated for the determination of scopoletin in dog plasma. The developed method was linear over the concentration range of 1-500 ng/mL. Extraction recovery, matrix effect, stability, accuracy, and precision for dog plasma samples were within acceptable limits of variation. Dogs were given a single intravenous dose of 1 mg/kg bw or an oral dose of 10, 25 or 50 mg/kg bw of scopoletin. Scopoletin had a short elimination half-life. Oral bioavailability was low (5.7-7.1%), and scopoletin showed dose-independent pharmacokinetic profiles in plasma over the dose range of 10-50 mg/kg bw. The predominant metabolic pathway in dog was glucuronidation.

1.6.1.2 Studies using herbal extracts containing scopoletin

Xia et al. (2007) developed and validated an HPLC method for quantification of scopoletin in rat plasma. Scopoletin was separated and purified from an ethanol extract of *E. obtusifolia* Benth. and its structure was confirmed by UV, IR, MS and NMR spectroscopy. The purity of the scopoletin was 99.5%. The LLOQ for scopoletin in plasma was 0.165 µg/mL. The LOD was 0.05 µg/ml at a signal-to-noise ratio of 3. Precision, accuracy, recovery, stability and matrix effect for rat plasma samples were within acceptable limits of variation. In the pharmacokinetic study, 5 rats (gender not stated) were fasted for 10 h with free access to water, then given a single oral gavage dose of scopoletin of 50 mg/kg bw. Blood samples were taken just before and 2, 5, 10, 15, 20, 30, 45, 60, 90 and 120 min after dosing. The results are shown in Figure 3. The time to peak dose (T_{max}) was obtained at 10 minutes and C_{max} was 8.2 $\pm$ 0.8 min. The $t_{1/2}$ was 14.1 $\pm$ 0.6 min.

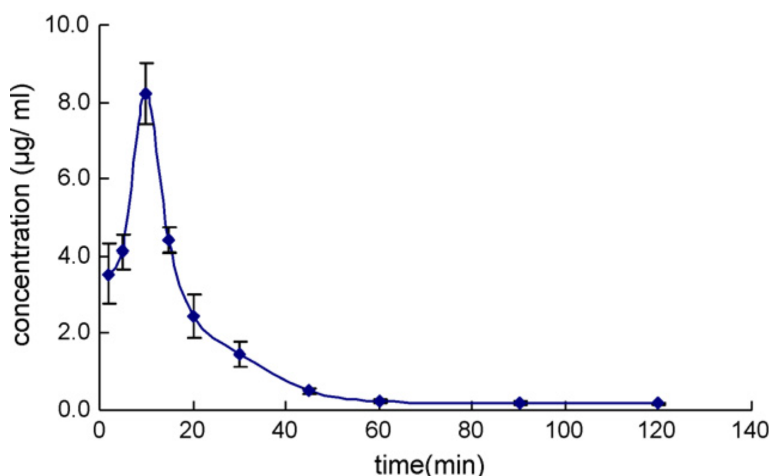

Fig. 3. Mean plasma concentration–time profile of scopoletin after oral administration of 50 mg/kg scopoletin in rats (Xia et al., 2007).

Xia et al. (2008) studied the absorption kinetics of scopoletin in rat stomach and intestines (Publication in Chinese, English abstract only seen). Rats (gender not stated) were cannulated for in situ recirculation. Scopoletin was measured by HPLC. The absorption rate in rat stomach at 2 h after administration was 76.31%. The absorption rates in colon, duodenum, ileum and jejunum were 46.25%, 40.54%, 38.21%, 32.77%, respectively. Intestinal absorption did not vary significantly between PH 6.0 and 7.4. The authors concluded that scopoletin was well absorbed in stomach and intestines and that absorption of scopoletin was a first-order process with a passive diffusion mechanism.

Chang et al. (2013) used LC-MS/MS to identify 8 coumarins, including scopoletin in rat plasma. Six male rats were given free access to food and water until 12h before dosing. They were given 6 g/kg orally of dried extract of Radix Angelicae Pubescentis, a traditional Chinese medicine that contains coumarins. Scopoletin concentration in the dried extract was 0.055 mg/kg. Blood samples were taken from the rats before dosing and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h after dosing. The LLOQ for scopoletin was 2.06 ng/mL. Accuracy, precision, recovery, stability and matrix effect for rat plasma were within acceptable limits. In the rats, scopoletin was below the LLOQ at all sampling times, in contrast to the other 7 coumarins, which were all detected.

Yi et al. (2014) developed a method to analyse multiple components of herbal medicines based on ultrahigh performance liquid chromatography coupled with diode array detection and quadrupole time-of-flight mass spectrometry (UPLC–DAD– QTOF-MS). The method was validated for selectivity, linearity, sensitivity, precision, accuracy, matrix effects, recovery and stability according to the US Food and Drug Administration (FDA) bioanalytical method validation guidelines (Food and Drug Administration, 2013). Recovery, stability, precision, accuracy, and matrix effects for rat plasma were within acceptable limits of variation. The LLOQ and LOD for scopoletin were 0.01 and 0.003 µg/mL, respectively. Tibetan "Snow Lotus" herb (*Saussurea laniceps,* SL) was selected as the test herb. The method was then applied to investigate the pharmacokinetics and metabolism of the components of SL extract in male rats. Scopoletin was one of the main components of the SL extract. For the pharmacokinetic study, 6 rats were fasted overnight, then given a single oral dose (1.0 g/kg) of SL extract and blood samples taken at 20, 30, 40, 60, 80, 100, 120, and 180 min post-dosing. For scopoletin, T_{max} was at 20 min, C_{max} was 5.55 $\pm$ 0.69 µg/mL, and $t_{1/2}$ was 34 min. In the metabolism study, a single oral dose of SL extract (1.0g/kg bw) was given to each of 2 rats. Blood samples were taken prior to dosing and at 20, 40, and 60 min post-dosing. Rat urine and faeces samples were collected from 0 to 24 h after oral administration of SL extract. Seventeen components were identified in blood, 10 components in urine and 2 components in faeces. Scopoletin was found in the SL extract, plasma, urine and faeces. In rat plasma, umbelliferone and scopoletin were the most abundant components of the SL extract found and they showed rapid absorption. The origin of the scopoletin in plasma was assumed to be either from direct absorption from the SL extract, or from Phase I metabolic hydrolysis of scopolin, the glycoside of scopoletin, which is also an original component of SL extract. Scopoletin glucuronide and scopoletin sulfate were found in plasma and urine and were confirmed as the main Phase II metabolites of scopoletin by oral administration of pure scopoletin to rats in a separate experiment. Scopoletin and its metabolites were also found in urine, with most of the scopoletin in the form of the two major metabolites. Traces of umbelliferone and scopoletin were found in the faeces.

Wang et al. (2018) administered Yin Chen Hao Tang (YCHT), a Chinese herbal medicine, to normal rats and rats with acute liver injury induced by carbon tetrachloride in a dose of 60 g/kg bw by gavage. The YCHT dose was equivalent to 0.49 mg/kg bw of scopoletin. Plasma pharmacokinetics of 8 bioactive components was measured using UHPLC–MS/MS. Blood samples were collected at 5, 10,

15, 30, 45, 60, 90, 120, 240, 480, 600, 720, and 1440 min. Recovery, stability, precision, accuracy, and matrix effects for rat plasma were within acceptable limits of variation. For scopoletin, there were no significant differences in PK values between normal and hepatic injury rats and scopoletin was rapidly eliminated from the plasma; $t_{1/2}$ was 3.6 and 2.2 h and T_{max} was 0.19 and 0.13 h, in control and hepatic injury rats, respectively.

Li Z et al. (2019) also used an LC-MS/MS method for determining the active components, including scopoletin, in rat plasma after administration of the traditional Chinese medicine, *Cortex periplocae*. The LLOQ, accuracy and precision of the assay in rat plasma were similar to those reported by Zeng et al. (2015) described above. Six male rats were fasted for 12 h with free access to water prior to dosing. Dried extract of *Cortex periplocae* was administered in a single oral dose of 18.9 g/kg, assayed to contain 7.09 mg scopoletin/kg bw. The blood samples were taken at frequent intervals after dosing (0.033, 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h), with fluid replacement (appropriate volume of physiological saline). The T_{max} for scopoletin was 0.10 ± 0.03 h and $t_{1/2}$ was 0.19 ± 0.08 h, which is shorter than that reported by Zeng et al. (2015) who gave scopoletin as such. The C_{max} was 177.83 ± 133.85 ng/mL, which is higher than that achieved by Zeng et al. (2015) with oral doses of 5 or 10 mg/kg bw of scopoletin as such. However, both studies show that scopoletin is rapidly absorbed and eliminated.

Zhou et al. (1997) studied absorption of scopoletin into rabbit serum following a single intra-muscular injection of Caulis Erycibes, a traditional Chinese medicine (publication in Chinese, English abstract only seen). Ten rabbits were used and scopoletin was measured by HPLC. The absorption of scopoletin was rapid with a T_{max} 1 of 8 min, C_{max} of 145.45 $\pm$ 47.65 ng/ml. The time to the second peak concentration (T_{max} 2) was 2.45 $\pm$ 1.79 h, C_{max} 2 = 48.66 $\pm$ 41.66 ng/ml.

1.6.2 Humans

Issell et al. (2008) investigated the pharmacokinetics of scopoletin as a bioactive marker of Noni fruit exposure in healthy human volunteers, aged 27 – 50 years, in Hawaii. Scopoletin was determined in plasma and urine by HPLC with photo diode array (PDA) and MS detection. No information was provided on the validation or performance of the assay for scopoletin. Capsules containing the 500 mg of whole freeze-dried fruit of Noni were orally administered at doses of 1,500 mg (3 capsules) to one male and two 2 female volunteers, 2,000 mg (4 capsules) to one male and two female volunteers, and 2,500 mg (5 capsules) to three male volunteers. Plasma and urine samples were obtained from each subject prior to dosing and at 0.5, 1, 2, 4 and 8 h after dosing. Excretion of scopoletin in urine was reported as the ratio of scopoletin to creatinine levels in each sample. The scopoletin content of the capsules of dried noni fruit was determined to be 23 mg, equating to ingestion of 69, 92 and 115 mg scopoletin for the three dose groups. After ingestion of capsules, scopoletin was the most prominent peak in plasma. There was rapid entry of scopoletin into plasma after ingestion of the capsules. T_{max} was 0.33, 0.5 and 0.5 h for the 69, 92 and 115 mg scopoletin groups, respectively, and the corresponding values for C_{max} were 3.14, 5.83 and 4.52 ng/mL. Scopoletin concentrations fell rapidly to around 2 ng/mL or lower between 2 and 8 h after dosing, but fluctuated during this time. The lack of a clear elimination phase precluded the determination of a $t_{1/2}$ for scopoletin in plasma, but it would have been at least 8 h. Detectable levels of scopoletin (0.04–3.46 ng/mL) were observed in the plasma of five of nine subjects prior to the administration of Noni extract and there was considerable variability between subjects. Urinary excretion of unchanged scopoletin after 8 h was estimated to be a small fraction (0.14–0.37%) of the orally ingested amount of scopoletin in all three groups. The estimated urinary clearance of scopoletin also varied significantly between subjects; mean values were 6.69 ± 2.75, 2.20 ± 1.2 and 5.46 + 3.59 mL/min kg for the 1500, 2000 and 2500 mg noni fruit doses, respectively. The authors commented that these values are equal to or greater than the glomerular filtration rate in healthy adults (1.8 mL/min kg), suggesting that scopoletin and/or its conjugates may be secreted as well as filtered into the urine. The authors concluded that scopoletin bioavailability appears to be low, with significant inter-subject variability and that scopoletin can be used as a relatively specific marker of Noni exposure in the blood and particularly in urine when its pharmacokinetics is considered appropriately.

In a subsequent Phase I clinical trial in ambulatory cancer patients, Issell et al. (2009) measured scopoletin concentrations in plasma and urine after administration of dried noni fruit in capsules. No information was provided on the validation or performance of the assay for scopoletin. Patients with malnourishment or significant organ failure were excluded in order to ensure absorption, distribution and elimination of noni components were not affected. Concentrations of scopoletin were determined by HPLC with PDA and MS detection. Scopoletin in urine was reported as the ratio of scopoletin to creatinine levels in each sample. Fifty-one patients were enrolled in the study. Twelve patients withdrew before the minimum doing period of 28 days was completed; withdrawals were due to a variety of reasons but none due to toxicity. Capsules of 500 mg, were administered at 7 levels (4, 8, 12, 16, 20, 24 or 28 capsules per day, giving doses of 2, 4, 6, 8, 10, 12 or 14 g per day), to a total of 39 patients, with 5 – 8 patients in dose levels 1–6 and 4 patients in dose level 7. Capsules were taken on an empty stomach at least 1-2 h before taking food. Starting with the lowest dose, 5 patients were followed for a minimum of 28 days before entering new patients into the next higher dose level. Baseline urine and blood samples were collected prior to starting noni, and then every four weeks while patients at the first five dose levels were on study. Urine samples were collected for 12 h overnight. Baseline plasma levels of scopoletin ranged from 0.0 – 42.8 nM/L. Baseline urine levels ranged from 0.2 – 4.9 nmol scopoletin/mg creatinine. After one month of dosing, plasma scopoletin concentrations were 55.0, 3.3, 64.9, 11.7, 52.1, 70.6 and 138.1 nM/L at dosing levels 1-7, respectively. Corresponding urinary concentrations were 1.7, 1.7, 2.4, 3.4, 6.4, 11.1 and 28.4 nmol/mg. There was a statistically significant, dose dependent appearance of scopoletin in plasma ($p<0.0001$) and urine ($p=0.03$). The authors commented that on the higher baseline concentrations of scopoletin for patients enrolled at higher dose levels, offering an explanation that with more public awareness as the study progressed, patients at the higher dose levels were ingesting marketed noni supplements at the time of study enrolment.

Juvonen et al. (2019) evaluated the metabolism of scoparone in human, mouse, rat, pig, dog, and rabbit liver microsomes in vitro and in humans in vivo. Human CYP2A13 exhibited the highest rate of scopoletin oxidation, followed by CYP1A1 and CYP1A2. Glucuronidation of scopoletin was catalysed by the human enzymes UGT1A1, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, and UGT2B17.

1.7 Biological and biochemical activities

The Noni plant, including its fruit has been used for traditional medicinal remedies by the Polynesians for hundreds of years (Mahadeva Rao et al., 2013; Ali et al., 2016). Numerous biological activities have been attributed to its major coumarin component, scopoletin (reviewed by Yin et al., 2010). Many of these activities have been demonstrated in vitro, but not all in vivo. The available data on biological and biochemical activities are summarised below, with more details on individual studies provided in the Annex. They are summarised, rather than described in full below because, as activities that are largely considered as potentially beneficial, they are only indirectly relevant to safety and toxicity.

1.7.1 Antioxidant activity

Antioxidant activity of scopoletin is well established from in vitro studies. In vitro, the effects are usually concentration-dependent. Reactive oxygen species (ROS) play an essential role in certain physiological processes, such as cell signalling, but if present in excess they can be damaging at the biochemical, genetic and cellular levels. Physiological and biochemical health depends on maintenance of an appropriate balance between ROS production and removal. ROS, such as superoxide, peroxides, hydroxyl radicals, and singlet oxygen, are free radicals produced endogenously in cells, that can readily interact with lipids, protein, DNA and RNA, causing damage and ultimately may cause cell death if they are not removed. Thus, the removal of excess ROS is an important defence activity in the body. Scopoletin enhances the activity of several endogenous enzymes that scavenge ROS, such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), glutathione peroxidase (GPx), and glutathione reductase (GR), present in high amounts in the liver, but also elsewhere. Scopoletin also has an inhibitory effect on the endogenous enzyme xanthine oxidase, whose activity generates ROS. There is controversy about whether oral consumption of antioxidants can have a beneficial effect on the body (Bast and Haenen, 2013; Juranek et al., 2013) and there is no direct evidence specific to scopoletin with respect to in vivo antioxidant activity.

1.7.2 Anti-inflammatory, anti-rheumatoid and anti-allergy activity

There is clear in vitro evidence that scopoletin inhibits synthesis of the inflammatory mediator nitric oxide (NO) in mouse macrophage and human mast cell lines. The inhibitory mechanism has been shown to be due to suppression of inducible NO synthase (iNOS) mRNA and iNOS protein. Scopoletin also inhibits production of other inflammatory mediators such as prostaglandin E2 (PGE2) and leukotrienes, and inhibits production of proinflammatory cytokines such as tumour necrosis factor-alpha (TNF-α) and interleukins (IL), IL-1β, IL-4, IL-5, IL-6 and IL-10, in vitro, in mouse macrophage and human mast cell lines and in human peripheral blood mononuclear cells, in a concentration-dependent manner. There is abundant evidence that scopoletin, in vitro, supresses activation of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and, in human mast cells, the mechanism was shown to be inhibition of the activation of NF-κB via inhibition of phosphorylation and degradation of the κB inhibitor, IκBα. NF-κB responds to harmful stimuli in cells, such as pathogens and increases in ROS and proinflammatory mediators. Increases in NF-κB activity control many of the genes involved in inflammatory reactions including stimulation of cytokine production.

Scopoletin also exhibits anti-inflammatory activity in in vivo, for example it reduces oedema and the levels of TNF-α and PGE2 in the mouse ear and hind paw oedema models. In the same models it increases the activity of antioxidant enzymes and lowers malondialdehyde levels, which is an indication of reduced damage to cellular lipids. It also reduces inflammation in mouse models of acute pancreatitis and lung injury and rat models of osteoarthritis, and prevents lipid disturbance in a rat model of liver injury.

1.7.3 Antinociceptive and analgesic activity

In in vivo mouse models, scopoletin, in the dose range 1-10 mg/kg bw, given by the intraperitoneal route, reduces pain responses.

1.7.4 Anticancer activity

There is conflicting in vitro evidence that scopoletin has anticancer activity. It has been tested against numerous human cancer cell lines in vitro; in some it has antiproliferative activity and induced apoptosis, generally in concentrations $\geq$100 µg/mL; in other cell lines it had no activity. Comparative studies with scopoletin derivatives or scopoletin analogues have shown that scopoletin is less active than many of these other structurally-related compounds. Some mechanistic studies in vivo, in a mouse carcinogen-induced skin cancer model, indicate that the antiproliferative/apoptotic activity may be due in part to preventing down-regulation of antiapoptotic signals, such as p53 and the caspases, which may be down-regulated in untreated tumour cells.

1.7.5 Antiangiogenesis

Antiangiogenic activity (inhibition of new blood vessel formation) may play a part in the demonstrated anti-inflammatory and antiproliferative activities of scopoletin. Reducing the blood supply to tumour tissue is a well-exploited treatment for certain cancers. Scopoletin has shown activity in in vitro tests for antiangiogenesis. In vivo, it reduced new blood vessel formation in the chick chorioallantoic membrane assay, in a mouse tumour xenograft assay, and in swollen paws in a rat arthritis model. It shows strong binding affinity toward known angiogenic factors, such as protein kinase, vascular endothelial growth factor A, and fibroblast growth factor 2, and downregulates their overexpression in the rat arthritis model.

1.7.6 Antihypertensive activity

In vitro, scopoletin reduced smooth muscle contractions in isolated rat blood vessel preparations and has inhibited noradrenaline-induced contractions in a variety of smooth muscle preparations from several species. In vivo, it reduced blood pressure in hypertensive rats and mice (0.01-5 mg/kg bw orally). The effect may be due in part to inhibition of intracellular calcium mobilization from noradrenaline-sensitive stores.

1.7.7 Antiulcerogenic activity

Scopoletin inhibited gastric acid secretion and reduced the formation of gastric ulcers in rat models of ethanol- or acetic acid-induced ulcers (0.5-50 mg/kg bw orally).

1.7.8 Liver effects

In adipocytes in vitro, scopoletin increased lipoprotein lipase activity (reduces triglycerides in vivo). In HEP2G cells modelled for hepatic steatosis, it reduced intracellular triglyceride, total cholesterol and expression of genes related to lipid metabolism. In vivo, in a mouse model of liver toxicity (alcohol + obesity), scopoletin (0.05% in the diet for 6 weeks) reduced plasma acetaldehyde, fatty acid, total cholesterol, triglyceride and insulin levels, hepatic lipid and droplets, and fasting blood glucose levels. It inhibited lipogenic enzyme activity and enhanced antioxidant enzyme activity. In a mouse model for hepatic steatosis (high-fat feeding diabetic type 1), scopoletin (0.01% in the diet for 11 weeks) lowered serum ALT, TNF-α and IL-6, and reduced hepatic lipid accumulation. It down-regulated hepatic gene expression of triglyceride and cholesterol synthesis and reduced inflammatory markers, suggesting it acts by inhibiting lipid biosynthesis and inflammatory pathways. In a rat model for liver toxicity (CCl_4 treatment), scopoletin (25 mg/kg bw orally for 7 days) reduced blood biomarkers of liver toxicity (ALT, AST) and bilirubin, raised serum protein, and protected against histological damage in the liver.

1.7.9 Neurological effects

Scopoletin has been shown to inhibit acetylcholine esterase (AChE) in a dose-dependent manner in vitro, and exhibit anti-adrenergic and anti-dopaminergic activities in vitro. There is inconsistent evidence on its ability to inhibit monoamine oxidase. In rat brain synaptosomes and hippocampal slices, scopoletin enhanced Ach release, shown to be mediated via nicotinic ACh receptors. It was protective against glutamate-induced toxicity in immortalised cells from mouse hippocampus.

In vivo in mice, scopoletin (2 mg/kg bw, s.c.) reduced age-associated deficits in object memory and dose-dependently (2.5-15 mg/kg bw, orally) attenuated scopolamine-induced amnesia, and inhibited AChE. In mouse models of depression, scopoletin had an antidepressant-like effect that was shown, with the use of receptor antagonists, to be dependent on serotonergic, noradrenergic and dopaminergic systems. In a mouse model for autoimmune encephalomyelitis to mimic multiple sclerosis, scopoletin (50 mg/kg bw, i.p., daily for 20 days) reduced severity of the disease and decreased inflammation and demyelination of central nervous system. In this model and in vitro, disease alleviation by scopoletin correlated with downregulation of major histocompatibility complex class II, CD80 and CD86, expressed on dendritic cells and with infiltration and polarization of encephalitogenic Th1/Th17 cells.

1.7.10 Antidiabetic effects

In vitro, in high-glucose-induced, insulin-resistant HepG2 cells, scopoletin stimulated reactivation of insulin-mediated protein kinase B phosphorylation, an effect blocked by a specific inhibitor of phosphatidyl inositol 3-kinase (PI3K). In adipocytes, it was less adipogenic than rosiglitazone, but did increase gene expression of PPARγ2, which regulates fatty acid storage and glucose metabolism, and increased adipocyte-specific fatty acid binding protein. This suggests scopoletin ameliorates insulin resistance in part by upregulating PPARγ2 expression.

In vivo, in rat and mouse models for diabetes, and in normal mice, scopoletin (10 mg/kg bw, orally) attenuated increases in blood glucose in oral glucose tolerance tests and reduced HB1C. In the rat, it reduced accumulation of in the liver of advanced glycation end products, which are biomarkers of diabetes, by a mechanism that suppressed protein tyrosine phosphatase 1B expression, thereby alleviating insulin resistance. In a rat model of pancreatic disease, scopoletin inhibited endoplasmic reticulum stress signaling by downregulating inositol requiring enzyme 1, protein kinase like endoplasmic reticulum kinase, and activating transcription factor 6 in pancreatic β-cells. Scopoletin also restored normal endoplasmic reticulum organelle ultrastructure. Using the same rat model, scopoletin lowered plasma glucose, insulin, and lipids, increased the activities of antioxidant enzymes, and reduced TBARS, lipid hydroperoxide, and protein carbonyl levels in plasma and pancreas. Mechanistic studies showed that scopoletin improved insulin signaling through activation of 5' AMP-activated protein kinase (AMPK), which activates glucose and fatty acid uptake in cells. A similar mechanism has been demonstrated for the action of scopoletin in increasing glucose uptake in mouse adipocyte-like cells in vitro.

1.7.11 Antifatigue activity

In mice given scopoletin (200-400 mg/kg bw/day for 6 weeks), gene expression analysis suggested the enhanced performance in weight-loaded swimming time to exhaustion was due to reduction in blood lactate and blood urea nitrogen, and increases in liver and muscle glycogen reserve through augmenting glucose metabolism, lipid catabolism, antioxidant defence responses, electron transport and energy production biomarkers.

1.7.12 Antithyroid activity

In thyroxine-treated rats given scopoletin (1 mg/kg bw/day, orally for 7 days), serum thyroid hormones were reduced, as well as glucose and hepatic glucose-6-phosphatase activity.

1.7.13 Melanin synthesis

Scopoletin (10-100 µM) in vitro increased melanin synthesis in murine melanoma cells, in a dose-dependent manner, by stimulating tyrosinase activity, the rate-limiting enzyme of melanogenesis. The mechanism was indicated to be by stimulation of production of microphthalmia-associated transcription factor, which controls tyrosinase expression via cAMP response element-binding protein phosphorylation.

1.7.14 Hypouricemic activity

Scopoletin (100-200 mg/kg bw, i.p.) inhibited activity of xanthine oxidase in liver homogenates of hyperuricemic mice, and in vivo decreased uric acid production and increased uric acid secretion in urine.

1.7.15 Antimicrobial activity

In vitro, scopoletin showed antimicrobial activity against a variety of Gram positive and Gram negative bacteria, and activity against the influenza virus and the leishmania parasite. It was not active against Plasmodium (malarial parasite).

1.7.16 Antiplatelet activity

Scopoletin had antiplatelet aggregation activity via down-regulation of thromboxane A2 production and intracellular Ca2+ mobilization, which are aggregation-inducing molecules produced in activated platelets.

1.8 Toxicity

1.8.1 Acute toxicity

The reported oral LD_{50} value for scopoletin in the rat is 3800 mg/kg bw (AIPTAK Archives, 1974). The intravenous LD_{50} value in the mouse is 350 mg/kg bw (Arzneimittelforschung, 1968).

1.8.2 Genotoxicity

Scopoletin reduced the mutagenic activities of cigarette smoke condensate and oral Swedish moist snuff (SNUS) in *Salmonella typhimurium* strains TA 98 and TA 100, in a dose-related manner, at 1-3 mg/plate, but cytotoxicity could not be excluded (Romert et al., 1994).

Scopoletin was shown to induce DNA strand breakage in the presence of copper ions (Ma et al., 2004). 500 ng of supercoiled pBR322 plasmid DNA was incubated with pure scopoletin at 37°C for 60 min, in the absence or presence of 20 µM Cu^{2+}. In the presence of copper ions, scopoletin concentrations of 250, 500 and 1000 µM caused concentration-dependent DNA strand breakage of 15, 22 and 36%, respectively. Scopoletin at 1000 µM in the absence of copper ions did not cause DNA strand breakage, neither was it active in the presence of iron ions (Fe^{2+}). It was suggested that reductive activation of copper by scopoletin was responsible for the cleavage activity.

Guardado Yordi et al. (2017) conducted in silico screening for clastogenic activity using a clastogenic QSAR model through the TOPological Sub-Structural MOlecular Design (TOPS-MODE) approach. This method uses the non-commercial software MODESLAB. In this model, coumarins with methoxy- and hydroxy groups were indicated as active. Of the 14 simple coumarins studied, scopoletin was predicted as the most active.

There are extensive genotoxicity data on coumarin itself, which have been reviewed elsewhere (IARC, 2000; EFSA, 2008; NSCFS, 2010; Api et al., 2020), from which it has been concluded that coumarin does not present a concern for genotoxicity.

1.8.3 Carcinogenicity

No animal bioassays for carcinogenesis of scopoletin have been located. Scopoletin has not been listed as a carcinogen by IARC, NTP, AGCIH or OSHA.

1.8.4 Male reproduction

Obidoa et al. (1999) treated 6 male guinea pigs with scopoletin, dissolved in 10% DMSO, at a single dose of 39.5 µg/kg bw per day by oral gavage for 15 consecutive days. The dose was stated to be "analogous to what is consumed by man" in a cassava-based diet. Six control animals were given the DMSO vehicle only. The animals were weighed at the start and the end of the 15-day dosing period. At the end of the treatment period the animals were anaesthetised and the testes, prostate and seminal vesicles were removed, but were not weighed. One g of tissue from each organ was weighed out, homogenised, and deproteinised, then fructose and citric acid contents were measured in testis and prostate plus seminal vesicles combined. Fructose and citric acid are produced in the male reproductive organs of many species in response to androgen stimulation, fructose providing the energy source for spermatozoa.

Mean starting weights were not comparable between the two groups (455.4 g versus 489.3 g in control and treated groups, respectively). Controls gained an average of 70.9 g (15.6%) during the 15 days, compared with 24.9 g (5.1%) in the treated group; this difference was statistically significant (p<0.01). Food and water intake were stated to remain normal during the study but no numerical data on these was provided. Citric acid and fructose levels in both the testis and prostate plus seminal vesicles were markedly reduced in the scopoletin-treated group, compared with controls, as shown in Table 2.

Table 2. Citric acid and fructose levels in male guinea pig reproductive organs after treatment with scopoletin

Organ	Citric acid (µg/mL)		Fructose (µg/mL)	
	Control	Scopoletin	Control	Scopoletin
Testis	14.30 ± 0.94	0.46 ± 0.18 *	1.25 ± 0.42	0.36 ± 0.16 #
Prostate & seminal vesicles	14.17 ± 1.45	2.90 ± 0.54 *	82.50 ± 7.28	2.09 ± 0.22 *

* P<0.01; # p<0.05

This is a poor study from the methodological point of view. There were only 6 animals per group, the testes, prostate and seminal vesicles were not weighed at the end of the study and the spermatozoa were not studied, which would have provided information on whether there were any functional consequences of the effects on fructose and citric acid. The reduction in body weight gain during the two weeks of scopoletin dosing was substantial, compared with the controls, suggesting there may have been considerable systemic toxicity. Thus, it is not possible to reach any conclusion on whether the effects on citric acid and fructose contents of the male reproductive organs were due to direct toxicity from scopoletin, or secondary to effects on body weight and systemic toxicity.

1.8.5 Neurotoxicity

In rats given scopoletin (0.7 µg/kg bw) plus cyanide (18 mg/kg bw) in the diet for 12 months, relative brain weights were reduced compared with controls from the third month onwards (Ezeanyika et al. 1999). There were no effects on lipid peroxide levels in the brain at 12 months. Histological examination suggested that scopoletin may be involved in the pathogenesis of neuropathy seen in cassava consuming populations. However, the majority of the literature relating to adverse neurological effects in cassava-consuming populations points to the cyanogenic glycoside content of cassava as causal (see review by Rivadeneyra-Domínguez & Rodríguez-Landa, 2020).

2. IDENTIFICATION OF DATA GAPS

From the abundant data on biological and biochemical activities (see section 1.7), which include a lot of mechanistic data, it is clear that scopoletin has a number of potentially beneficial properties, such as antioxidant, anti-inflammatory and antiproliferative activity, that might serve to reduce the likelihood of toxicity. However, given its ability to influence a variety of sub-cellular signalling pathways, it would be prudent to assume that scopoletin could also produce adverse effects at high doses.

The data on **toxicokinetics** (see section 1.6), include both human and rat studies. They are adequate methodologically and give a clear picture of the absorption, distribution, metabolism and excretion of scopoletin. Further studies would not be necessary.

From the review of the few available toxicological studies (see section 1.8), it is evident that there are almost no data from good-quality studies that are appropriate for risk assessment.

- It has low **acute toxicity**, but such data are not very relevant for risk assessment of regularly consumed substances and give no indication about possible effects from longer durations of exposure. Further studies on acute toxicity would not be of use.
- There are no conventional **repeated-dose toxicity** studies on scopoletin of any duration. The absence of such a study, or studies, is a major data gap and would be essential for risk assessment.
- There are no **genotoxicity** studies that would satisfy current-day, minimal requirements for assessing potential for genotoxicity. Such studies would be essential for risk assessment.
- There are no **carcinogenicity** studies on scopoletin. Coumarin itself is regarded as a non-genotoxic carcinogen in rodents, causing tumours in liver and lung in rats and mice, but the mechanism is thought to be attributable to hindering of 7-hydoxylation such that metabolism then goes down a 3,4-epoxidation pathway (Lake, 1999; Hsieh et al, 2019). Scopoletin is already 7-hydroxylated and so unlikely to exhibit carcinogenicity via this mechanism. Given this background and the fact that scopoletin has low oral bioavailability and is eliminated from the body very rapidly, provided that an adequate package of genotoxicity studies did not indicate any concern for genotoxicity, then carcinogenicity studies may be considered unnecessary.
- There is one study on male **reproductive effects** in the guinea pig, but the methodology is poor and the endpoints studied are rarely used in risk assessment. There are no other studies on reproductive organs, and no studies on reproductive capacity in adult animals, or on prenatal and postnatal development. Such studies would be essential for risk assessment.
- For **neurotoxicity**, there is one limited study available with a research methodology that indicates an adverse effect but does not allow risk assessment. Given the demonstrated potential for effects of scopoletin on enzymes and neurotransmitters that are important in the central and peripheral nervous system functioning (see section 1.7), the lack of more conventional in vivo neurotoxicity studies is a data gap. However, neurotoxicity studies are not routinely performed on food ingredients and are often only considered necessary if there are indications of neurotoxicity (clinical signs, behavioural effects or histopathological effects) in other repeated-dose studies. Given that inhibition of acetylcholinesterase is potentially toxic, it would be prudent to measure brain, red blood cell and plasma cholinesterase in rats in a repeated dose -study.
- There are no studies on **immunotoxicity**. Coumarin itself is a weak skin sensitizer, but is not phototoxic or photo-allergenic (Api et al., 2019). Studies on oral immunotoxicity are rarely available for food ingredients and would not be a priority for risk assessment.

3. ADDRESSING OF DATA GAPS

Considering the above analysis of the gaps in the toxicological data available on scopoletin, the following studies can be recommended as the minimum necessary to address these data gaps and allow risk assessment, including data necessary to underpin any proposal for an upper limit for scopoletin in noni juice.

1. A repeated-dose toxicity study in the rat, minimum duration of 90-days, to include measurement of brain, red blood cell and plasma acetylcholinesterase at termination.
2. A battery of *in vitro* genotoxicity tests to cover the endpoints of gene mutation, Structural (clastogenicity) and numerical (aneuploidy) chromosome aberrations. A bacterial reverse mutation assay and an *in vitro* micronucleus assay would suffice to cover these three endpoints. In the case of positive results *in vitro*, an appropriate *in vivo* study, to assess whether the genotoxic potential observed *in vitro* is expressed *in vivo*, would be necessary.
3. A test for reproductive toxicity in the rat, such as a multigeneration study or an extended one-generation reproduction study would be necessary.
4. A developmental toxicity study in the rat would be necessary. If negative, then a study in the rabbit might also be necessary.

All the above studies should be conducted in accordance with international testing guidelines, such as the relevant OECD test guidelines, which are available for all the proposed studies, and in accordance with international guidelines for Good Laboratory Practice (GLP).

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ANNEX

Table of biological and biochemical activities of scopoletin

Biological/biochemical activity	Findings	References
Antioxidant	Scopoletin had some inhibitory activity on xanthine oxidase (XO). XO generates reactive oxygen species (ROS).	Chang et al. 1994
	Scopoletin enhanced activity of endogenous antioxidants including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH), in rat hepatocytes in vitro.	Kang et al. 1998
	Scopoletin, isolated from *Sinomonium acutum*, scavenged superoxide anions in xanthine/xanthine oxidase reaction system in vitro in a concentration-dependent manner but did not inhibit xanthine oxidase.	Shaw et al. 2003
	Scopoletin, isolated from rhizomes of *Curcuma* manga, showed antioxidant activity in vitro.	Abas et al. 2005
	Scopoletin identified as one of main phenolic antioxidants in *Pedilanthus tithymaloides* (from the Euphorbiaceae family) used in traditional Cuban medicine.	Abreu et al. 2008
	Scopoletin (50 µg/mL) inhibited *tert*-butylhydroperoxide (*t*-BHP)-induced generation of ROS and prevented decreases in antioxidant enzymes, SOD, CAT, glutathione peroxidase (GPx) and glutathione reductase (GR) caused by *t*-BHP in HepG2 cells (human liver adenocarcinoma cell line).	Noh et al. 2010
	Scopoletin (50 µg/mL) prevented decreases in CAT, SOD, GPx and GR in ethanol-treated HepG2 cells.	Noh et al. 2011
	Scopoletin showed antioxidant activity in vitro in 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical assay, in scavenging hydrogen peroxide, superoxide, and hydroxyl radicals, and in ferrous ion chelating assay.	Malik et al. 2011
	Scopoletin showed high antioxidant activity in the DPPH free radical assay in vitro (IC_{50} 1.24 µg/mL).	More et al.2012
	Scopoletin, isolated from leaves of *Canarium patentinervium* Miq. (Burseraceae Kunth.), showed antioxidant activity in vitro in DPPH assay, 2,2’-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS) assay, Ferric Reducing Ability of Plasma (FRAP) assay, and β-Carotene Bleaching assay.	Mogana et al. 2013
	Scopoletin, isolated from *Foeniculum vulgare*, a traditional medicine, showed antioxidant activity in DPPH and ABTS assays.	Yang et al. 2015
	Scopoletin had moderate antioxidant activity in DPPH assay (54% at 100 µM).	Ibrahim et al. 2018
Anti-inflammatory **Anti-rheumatoid** **Anti-allergy**	Scopoletin, isolated from *Artemisia feddei,* inhibited synthesis of the inflammatory mediator, nitric oxide (NO), in a dose-dependent manner in RAW 264.7 murine macrophages stimulated with interferon-gamma (IFN-gamma) plus lipopolysaccharide (LPS). The inhibition was due to suppression of inducible NO synthase (iNOS) mRNA and iNOS protein.	Kang et al. 1999

Biological/biochemical activity	Findings	References
	Scopoletin, isolated from bark *Fraxinus rhynchophylla*, inhibited NO synthesis in a dose-dependent manner in RAW 264.7 murine macrophages stimulated with IFN-gamma plus LPS. Inhibition of NO synthesis was accompanied by decreased iNOS protein.	Kim et al. 1999
	Scopoletin, extracted from *Eupatorium buniifolium*, inhibited oedema by 60% in response to inflammation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in the TPA-mouse ear model at 1mg/ear.	Muschietti et al. 2001
	Scopoletin (1-50 µg/mL) inhibited, in a dose-dependent manner, production of prostaglandin E_2 (PGE2), and the pro-inflammatory cytokines, tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1b, and IL-6 in RAW 264.7 cells stimulated with IFN-g plus LPS in vitro. It also inhibited expression of cyclooxygenase enzyme COX-2 but not COX-1.	Kim et al. 2004
	Pre-treatment with scopoletin (0.01-0.2 mM) inhibited production of TNF-α, IL-6 and IL-8 in a dose-dependent manner in a human mast cell line (HMC-1) stimulated to produce cytokines by phorbol 12-myristate 13-acetate (PMA) plus A23187. Mechanism shown to be inhibition of NF-κB activation via inhibition of phosphorylation and degradation of IκBα.	Moon et al. 2007
	Scopoletin (100-200 mg/kg bw i.p.) inhibited oedema, decreased vascular dye leakage and reduced overproduction of PGE2 and TNF-α in croton oil-induced mouse ear oedema, consistent with reduced myeloperoxidase (MPO) activity and polymorphonuclear (PMN) infiltration. It attenuated hind paw oedema induced by carrageenan in mice, and lowered MPO activity and malondialdehyde (MDA) level in paw tissues (100-200 mg/kg bw i.p.).	Ding et al. 2008
	Scopoletin (25-100 µM) inhibited release of IL-6 and TNF in a dose-dependent manner in LPS-stimulated rat alveolar macrophage cell line NR8383.	Bissonnette et al. 2009
	Using the *λ*-carrageenan (Carr)-induced model of paw oedema in mice, scopoletin (5 -10 mg/kg bw i.p.), extracted from *Crossostephium chinensis*, reduced Carr-induced oedema, increased SOD, CAT and GPx, and attenuated MDA in the oedematous paw. It decreased NO, TNF-*α* and PGE2 levels in serum after Carr injection and decreased Carr-induced iNOS and COX-2 expressions in the oedematous paw.	Chang et al. 2012
	In the mouse air pouch model for acute gout with inflammation induced by injection of urate crystals, scopoletin (purity >98%), isolated from the stems of *E. obtusifolia* Benth, inhibited MPO production and reduced leukocyte, neutrophil and monocyte infiltration at 100 and 200 mg/kg bw i.p.. In vitro, scopoletin suppressed IL-1β, TNF-α, IL-6, PGE2 and NO. Regulation by scopoletin at the transcriptional level of these mediators was via suppression of NF-κB activation and blockade of MAPK signal pathway.	Yao et al. 2012

Biological/biochemical activity	Findings	References
	Scopoletin inhibited PMA/ionomycin-induced IL-4, IL-5, and IL-10 production in mouse EL-4 T cells. It enhanced inhibitory action of PMA/ionomycin on interferon-γ (IFN-γ) expression and downregulated expression of transcription factors NFAT (nuclear factor of activated T cells) (NFAT) and GATA-3. The downregulation depended on protein kinase C (PKC) attenuation, suggesting that anti-allergic properties of scopoletin might be mediated by downregulation of cytokine expression in Th 2 cells.	Cheng et al. 2012
	Scopoletin, isolated from *Canarium patentinervium* Miq., inhibited activity of 5-lipoxygenase in vitro (IC_{50} 1.8 µM), a key enzyme in arachidonic acid metabolism responsible for formation of pro-inflammatory leukotrienes.	Mogana et al. 2013
	In fibroblast-like synoviocytes (FLS) from rats with adjuvant-induced arthritis, scopoletin (15, 30, 60 µM) moderately inhibited FLS proliferation in vitro, markedly reduced IL-6 production at mRNA and protein levels, and inhibited phosphorylation of p38 mitogen-activated protein kinase, extracellular signal-regulated kinase (ERK), protein kinase C (PKC) and cAMP response element binding protein (CREB). This suggests that scopoletin exerts anti-inflammatory effects in rheumatoid arthritis by suppressing IL-6 production from FLS via MAPK/PKC/CREB pathways.	Dou et al. 2013
	Scopoletin, isolated from roots of *Carissa carandas* (L.), inhibited production of TNF-α and IL-1β (by c.50% at 30 µM), and inhibited production of NO in PLS-stimulated cells in vitro (IC_{50} 24.6 µg/mL).	Galipalli et al. 2014
	Scopoletin (0, 0.2, 1, 5, 25 µM) had no effect on NO production or iNOS expression, or on IL-6, IL-1β, and TNF-α levels in LPS-stimulated RAW 264.7 murine macrophages.	Ju et al. 2015
	In a rat model of chronic alcohol ingestion, inducing disturbance of hepatic lipid homeostasis and inflammation in white adipose tissue (WAT), adding scopoletin at 0.001 and 0.005% to the liquid diet for 8 weeks decreased serum triglyceride, TNFα, IL-6, and hepatic and WAT lipid levels. It also reduced expression of hepatic genes TLR4, MyD88, TRIF, which activate NF-kB, that in turn causes increased production of pro-inflammatory cytokines.	Lee & Lee 2015
	Scopoletin, isolated from *Foeniculum vulgare*, inhibited production of IL-6 and TNF-α in LPS-stimulated RAW 264.7 murine macrophages through blockade of the IκB kinase (IKK)/inhibitor of kappa B (IκB)/NF-κB pathway.	Yang et al. 2015
	Scopoletin inhibited PGE2 production in LPS-stimulated RAW 264.7 murine macrophages in a dose dependent manner (10-100 µM).	Cai et al. 2016
	Svcopoletin and umbelliferone isolated from *Glehniae Radix cum Rhizoma* (Hamabofu in Japanese) suppressed production of inflammatory mediator NO in rat hepatocytes in vitro.	Kamino et al. 2016
	Scopoletin (0.5, 2, 5 and 20 µg/mL), isolated from *A. annua*, inhibited NO production by 65-98% in LPS-activated RAW 264.7 murine macrophages.	Chougouo et al. 2016

Biological/biochemical activity	Findings	References
	In mouse model of pleurisy, scopoletin (1 mg/kg bw) reduced cell migration and exudation into pleural fluid, decreased MPO activity, adenosine-deaminase activity and NO, TNF-α, and IL-1β levels. It reduced p65 and p38 phosphorylation in mouse lungs. Effect attributed to ability of scopoletin to inhibit phosphorylation of NF-κB and p38 MAPK.	Pereira Dos Santos Nascimento et al. 2016
	Scopoletin bound to NF-κB and its regulator IκB. Scopoletin (20 or 40 µM) activated NF-κB in a SEAP-driven NF-κB reporter cell line.	Seo et al. 2016
	Scopoletin (20 µM), isolated from *Angelica dahurica*, reduced release of histamine from rat basophilic leukaemia (RBL)-2H3 cells used for in vitro studies of mast cell-mediated allergic inflammation, but had no effect on TNF-α, IL-1β or IL-4 secretion.	Li & Wu 2017
	Scopoletin (500 µM), isolated from *B. rupestris* leaves, inhibited histamine release from RBL-2H3 cells and reduced release of the inflammatory enzyme elastase from human neutrophils.	Thabet et al. 2018
	Scopoletin, isolated from isolated from *Artemisia capillaris*, markedly decreased MMP-1, IL-1α and TNFα mRNA expression in UV-exposed, human HaCaT-stimulated keratinocytes, without causing cytotoxicity, in a dose-dependent manner, by reducing phosphorylation of p38 MAPK.	Kim et al. 2018
	Scopoletin (1-100 µM) showed moderate anti-inflammatory activity in vitro (stabilisation of red blood cell membrane in hypotonic conditions), and reduced NFκB, p65, TNF-α and IL-6 in human peripheral blood mononuclear cells after PHA-challenge.	Ibrahim et al. 2018
	Scopoletin (1 mg/kg bw i.p.) attenuated severity of acute pancreatitis, induced in mice by cerulein, and reduced associated lung injury and reduced pancreatic and lung proinflammatory cytokines (IL-1β, TNF-α) and hydrogen sulfide. It also inhibited cerulein-induced NF-κB activation in pancreas and lung and reduced cerulein-induced mast cell activation, as shown by reduced monocyte chemoattractant protein 1, IL-33, and preprotachykinin A expression (encodes neuropeptide substance P) in pancreas and lungs.	Leema & Tamizhselvi 2018
	In LPS + INFγ-activated RAW 264.7 murine macrophages, scopoletin (10-100 µM) reduced NO production but did not protect against cell death.	Bakondi et al. 2019
	Scopoletin (1 or 5 µM), isolated from *Erycibe schmidtii*, functionally reduced proliferation of bone marrow immature dendritic cells by more than 50%. However, it exhibited no effect on the phagocytosis or survival of these cells in vitro.	Ren et al. 2019
	Scopoletin (40 µM), isolated from roots of *Eurycoma longifolia* Jack, reduced NO and protein production and reduced expression of IL-6, NF-κβ and iNOS in LPS-induced RAW264.7 murine macrophages.	Ruan et al. 2019

Biological/biochemical activity	Findings	References
	A scopoletin-standardized (200 or 400 mg/kg bw) extract of *Morinda elliptica* leaf extract was investigated in ex vivo explant culture and preclinical rodent model for osteoarthritis (OA). The extract suppressed glycosaminoglycan and NO release from cartilage explants in the presence of IL-1β. In vivo, it dose-dependently reduced serum inflammation biomarkers, and increased bone formation biomarkers to near normal levels in the OA-induced rats.	Wan Osman et al. 2017, 2019
Antinociceptive Analgesic activity	Scopoletin (0.001-10 mg/kg bw i.p.), isolated from *Polygala sabulosa A*. W. Bennett, exhibited dose-related antinociceptive effects against acetic acid-induced visceral pain in mice.	Meotti et al. 2006
	In glutamate-induced, paw licking model in mice, scopoletin (10 mg/kg, i.p.) inhibited the nociceptive response by 46%.	Ribas et al. 2008
	Scopoletin (1-10 mg/kg bw i.p.) inhibited the acetic acid-induced writhing response and late phase response to formalin-induced pain in mice.	Chang et al.2012
Anticancer activity	Scopoletin showed antitumour activity in vivo in the mouse P-388 lymphocytic leukaemia system.	Cassady et al. 1979
	Scopoletin was cytotoxic in human small cell lung carcinoma cell line (GLC4), and human colorectal cancer cell line (COLO 320).	Kolodziej et al. 1997
	Scopoletin inhibited cell proliferation in human cell lines for androgen-independent prostate cancer (PC3), lung cancer (PAA) and cervical cancer (HeLa) (IC_{50}s 154-294 µg/mL). In PC3 cells, inhibition was concentration and time-dependent and 2-35% of cells showed apoptosis at 100-400 µg/mL.	Liu et al. 2001
	Scopoletin, isolated from *Simarouba glauca* twigs, did not inhibit cell proliferation in human cell lines for epidermoid cancer (KB, now known to be HeLa (cervical cancer) subline), or lung cancer (Lu1).	Rivero-Cruz et al. 2005
	Scopoletin (0.1-0.5 mg/mL) inhibited cell proliferation and induced apoptosis in dose-dependent manner in human promyeloleukemic (HL-60) cells. It activated a signal cascade including the heterodimeric, redox-sensitive transcription factor NF-κB, with upregulation of NF-κB translocation to the nucleus by increase of Iκ-Bα degradation. Scopoletin also activated caspase-3, suggesting that it induces NF-κB activation, causing activation of caspase-3, degradation of PARP, eventually leading to apoptotic cell death. *Note that upregulation of NF-κB is contrary to the evidence from anti-inflammatory studies.*	Kim et al. 2005
	Scopoletin, isolated from *Tilia cordata* Mill. flowers, inhibited cell proliferation and induced apoptosis in BW 5147 mouse lymphoma cells.	Barreiro Arcos et al. 2006

Biological/biochemical activity	Findings	References
	Scopoletin inhibited cell proliferation in human CCRF-CEM leukaemia cells (IC_{50} 2.6 µM) and in multidrug resistant subline, CEM/ADR5000 (IC_{50} 1.6 µM).	Adams et al. 2006
	Scopoletin did not inhibit proliferation in human cell lines for lung cancer (Lu1), androgen-sensitive human prostate cancer (LNCaP), or breast cancer (MCF-7).	Kim et al. 2006
	Scopoletin did not inhibit proliferation in human cell lines for lung cancer (A549), ovarian cancer (SK-OV-3), melanoma (SK-MEL-2), or colon cancer (HCT15) (ED_{50}s>5 µg/mL).	Kwak et al. 2009
	Activity of a close, synthetic structural analogue of scopoletin, 4-methyl-7-hydroxycoumarin, was investigated in DMBA-induced skin cancer in mice. 50 and 100 mg/kg bw per day orally for 24 weeks reduced papillomas and cytogenetic abnormalities. In mice treated with carcinogen only, DMBA induced toxicity and over-expression of aryl hydrocarbon receptor (AhR), IL-6, proliferating cell nuclear antigen (PCNA), Akt, Bcl-2, Bcl-xL, and NF-kB, and down-regulated p53, and the antiapoptotic signals Bad, Bax, Apaf, Cytochrome-c, Caspase-3 and Caspase-9. In mice treated with the scopoletin analogue, the expressions of these proteins and toxicity biomarkers were reverted.	Battacharrya et al. 2009
	In a study analogous to the one above, scopoletin, separated from an extract of the plant *Gelsemium sempervirens*, was given in daily oral doses of 50 or 100 mg/kg bw. In mice treated with carcinogen only, DMBA induced toxicity and over-expression of AhR, CYP1A1, PCNA, Stat-3, survivin, MMP-2, cyclin D1 and c-myc and down-regulation of p53, caspase-3 and TIMP-2. In mice treated with scopoletin, the expressions of these proteins and toxicity biomarkers were reverted.	Battacharrya et al. 2010
	In human melanoma cell A375, nanoencapsulated scopoletin, isolated from *Gelsemium sempervirens*, down-regulated cyclin-D1, PCNA, survivin and Stat-3, and up-regulated p53 and caspase-3, that in turn induced greater apoptosis compared with unencapsulated scopoletin.	Khuda-Busch et al. 2010
	Scopoletin, isolated from leaves of *Morinda citrifolia* Linn., did not show a strong inhibitory effect against KB (human epidermoid carcinoma), HeLa (human cervical carcinoma), MCF-7 (human breast carcinoma) and HepG2 (human hepatocellular carcinoma) cell lines (IC_{50}s 103->600 µg/mL).	Thani et al. 2010
	Scopoletin, isolated from *Heteroplexis micocephal*, was not cytotoxic against human stomach cancer cell line (BGC-823) or human lung cancer cell line (A549).	Fan et al. 2011
	A number of derivatives of scopoletin had higher inhibitory potency on growth than scopoletin itself in mammary (MCF-7 and MDA-MB 231) and colon (HT-29) carcinoma cells (IC_{50}s <20 µM compared with scopoletin >100 µM).	Liu et al. 2012
	A series of 24 synthetic scopoletin derivatives were tested alongside scopoletin in tests for antiproliferation in 14 human cancer cell lines. Several of the derivatives were more active than scopoletin.	Cai et al. 2013

Biological/biochemical activity	Findings	References
	Scopoletin, isolated from roots of *Decaschistia parviflora*, showed some cytotoxicity against KB cells (IC50 66 µM), but was inactive against MCF-7 or NCI-Hi87 (human small cell lung cancer) cell lines.	Wongsa et al. 2013
	Scopoletin (20 µg/mL), isolated from leaves of *Micromelum integerrimum*, did not show cytotoxicity against HeLa, A549, or BGC-823 human cancer cell lines.	Wang et al.2014
	A number of scopoletin-cinnamic acid hybrids exerted much higher inhibition of growth in human tumour cell lines (MCF-7, MDA-MB-231, A549, HCT-116, and HeLa), compared with scopoletin itself.	Li et al. 2015
	Scopoletin, isolated from *Artemisia dracunculus*, inhibited cell proliferation in oesophageal cell line (EC-109) but other isolates were more potent.	Hong & Ying 2015
	Using an NCI panel of 56 human tumour cell lines (from lung, kidney, brain, colon, leukaemia, ovarian, melanoma and prostate cancers), scopoletin showed favourable antiproliferative activity against tumour cell types with ATP-binding cassette (ABC)-transporter expression.	Seo et al. 2016
	Scopoletin was cytotoxic (IC_{50} 8.6 µM) against human bladder carcinoma cell line (EJ) but not against human hepatocellular carcinoma cell line (SMMC-7721).	He et al. 2017
	Scopoletin did not inhibit proliferation in human cell lines from colorectal cancer (HCT-116, SW620), liver cancer (Hun7, HEPG2), breast cancer (MDA-MB-231, MCF-7), or lung cancer (A549), nor in normal human cells HFL-1, whereas some synthetic scopoletin derivatives, especially scopoletin-isoxazole, were antiproliferative in cancer cells but not normal cells.	Shi et al. 2017, 2018
	Scopoletin inhibited the enzyme NAD(P)H:quinone oxidoreductase-1 (NOQ1), which is over-expressed in cholangiocarcinoma (CCA), in human-derived KKU-100 CCA cells, and inhibited cell migration. Antimigration effect associated with decreased ratio of matrix metalloproteinase 9/tissue inhibitors of metalloproteinases 1 (MMP9/ TIMP1) mRNA.	Khunluck et al. 2018
	Scopoletin (10 or 30 µM) did not inhibit proliferation in human gynaecological cancer cell lines (SiHa, HeLa, MDA-MB-231).	Tastan et al. 2019
	Scopoletin showed moderate cytotoxicity against A375 (IC_{50} 117 µM) and HeLa (IC_{50} 74 µM) cells, but was inactive against SMMC-7721, SGC-7901 (human gastric cancer), HCT-116 and MDA-MB-231 cells.	Sheng et al. 2019
	Scopoletin (3.5 -100 µM) decreased cell viability of HeLa cells and of normal HCvEpC cervical cells (2.5-100 µM) and inhibited migration of HeLa cells. In cervical cancer cell lines (DoTc2, SiHa, HeLa, C33A), scopoletin caused dose-dependent inhibition of growth (IC_{50}s 7.5-25 µM). Mechanism was blocking of PI3K/AKT signal transduction pathway by inhibition of phosphorylation of PI3K and AKT proteins.	Tian et al. 2019

Biological/biochemical activity	Findings	References
Antiangiogenic activity	In rat model of adjuvant-induced arthritis, scopoletin (50 or 100 mg/kg i.p.) reduced both inoculated and non-inoculated paw swelling and articular index scores, and increased mean body weight of adjuvant-induced arthritic rats. The higher dose of scopoletin showed near-normal histological architecture of the joints and reduced new blood vessel formation in synovial tissues. Scopoletin downregulated overexpression of vascular endothelial growth factor, basic fibroblast growth factor and IL-6 in synovial tissues.	Pan et al. 2010
	Scopoletin, isolated from *Morinda citrifolia* L., showed antiangiogenic activity in vivo in chick chorioallantoic membrane assay.	Beh et al. 2012
	A series of 24 synthetic scopoletin derivatives were tested alongside scopoletin in endothelial cell migration assay and tube formation study tests for antiangiogenesis. Several of the derivatives were more active than scopoletin.	Cai et al.2013
	Scopoletin, isolated from tree tobacco *Nicotiana glauca*, suppressed sprouting of microvessels in rat aortic explants (IC_{50} 0.06 µM). In vivo, scopoletin (100 and 200mg/kg bw) inhibited (59.72 and 89.4%, respectively) vascularization in matrigel plugs implanted in nude mice and in tumour xenograft model, it inhibited tumour growth. Tumour histology showed drastic reduction of vascularization. Immunostaining of CD31 and NG2 receptors in histological sections confirmed antivascular effect of scopoletin in tumour vasculature. Scopoletin showed strong ligand affinity and binding energies toward the following angiogenic factors: protein kinase (ERK1), vascular endothelial growth factor A (VEGF-A), and fibroblast growth factor 2 (FGF-2).	Tabana et al. 2016
Antihypertensive activity ***Smooth muscle spasmolytic***	Scopoletin, isolated from fruits of *Tetrapleura tetraptera* T*AUB* (Mimosaceae), reduced amplitude and frequency of spontaneous, myogenic, rhythmic contractions, and exogenous noradrenaline (NA)-evoked contractions of the rat isolated portal vein. Also relaxed and inhibited electrically-induced or NA-induced contractions of various smooth muscles in cat, rabbit, guinea pig and chick.	Ojewole & Adesina 1983
	Scopoletin, isolated from the roots of *Brunfelsia hopeana* Benth. (Solanaceae), inhibited contractions in isolated rat aortic rings (26-520 µM) and antagonized transient contractions in Ca($^{2+}$)-free media induced by noradrenaline, in a dose-dependent manner (IC_{50} 300 µM). The effect was attributed in part to inhibition of intracellular calcium mobilization from noradrenaline-sensitive stores.	Oliveira et al. 2001
	Morinda citrifolia fruit ethanolic extract, containing 0.46 + 0.05% scopoletin, reduced blood pressure in hypertensive rats.	Wigati et al. 2017

Biological/biochemical activity	Findings	References
	Scopoletin, isolated from *Malva parviflora*, given 0.01 – 5 mg/kg bw orally, 24h, 18h and 1h before hypertensive challenge, reduced blood pressure in mice in a dose-related manner.	Lagunas-Herrera et al. 2019
	Scopoletin (30 µM) caused 90% vasorelaxation in rat main mesenteric artery, but was not as active as a number of chromeno-coumarin hybrids synthesised from scopoletin.	Singh et al. 2020
Antiulcerogenic activity	Scopoletin (0.5 and 1 mg/kg bw) given intra-duodenally or orally, reduced gastric acid secretion and inhibited gastric ulcer formation by 67-70% in ethanol- or acetic acid-induced ulcer models in rats.	Mahattanadul et al. 2011
	Scopoletin and scopolin, isolated from *Convolvulus austro-aegyptiacus*, (50 mg/kg bw) protected against ulcers by 16.7% and 90.8%, respectively, in ethanol- induced ulcer model in rats.	Awaad et al. 2015
Liver effects ***Lipid disturbances***	Scopoletin increased lipoprotein lipase (LPL, which removes plasma triglycerides) activity in vitro in 3T3-L1 adipocytes in dose- and time-dependent manner. It did not release LPL from the adipocyte membrane but increased the LPL mRNA, suggesting transcriptional control.	Yang JY et al. 2007
	In a model of alcohol-induced liver toxicity in obese mice, concurrent scopoletin in the diet (0.05% w/w daily for 6 weeks) reduced plasma acetaldehyde, fatty acid, total cholesterol, triglyceride and insulin levels, hepatic lipid and droplets, and fasting blood glucose levels that were increased by alcohol. Scopoletin activated hepatic AMP-activated protein kinase (AMPK) and inhibited ACC and SREBP-1c and the activities of lipogenic enzymes, such as FAS, PAP and G6PD, compared to the alcohol control. It also inhibited hepatic CYP2E1 activity and protein levels but elevated activities of SOD, CAT, GSH-Px and GST and levels of GSH, compared to the alcohol control group. It lowered hepatic lipid peroxide.	Lee et al. 2014
	In the carbon tetrachloride-induced liver toxicity model in the rat, scopoletin (25 mg/kg bw orally for 7 days) reduced blood biomarkers of liver toxicity, SGOT (serum glutamic oxaloacetic transaminase or AST) and SGPT (serum glutamic pyruvic transaminase or ALT), and bilirubin, and raised serum protein. Also protected against histological damage showing normal hepatic cords, absence of necrosis, and fatty infiltration.	Goudar et al. 2015
	In a model for hepatic steatosis and inflammation using high-fat diet fed type 1 diabetic mice, scopoletin in the diet (0.01% w/w daily for 11 weeks) lowered serum ALT, TNF-α and IL-6, and reduced hepatic lipid accumulation. It down-regulated hepatic gene expression of triglyceride (PPARγ, Plpp2, and Dgat2) and cholesterol (Hmgcr) synthesis and reduced inflammatory markers (TLR4, Myd88, Nfkb1, TNF-α, and IL-6), while up-regulating Cyp7a1 gene.	Choi et al. 2017

Biological/biochemical activity	Findings	References
	It also inhibited hepatic fatty acid synthase and phosphatidate phosphohydrolase activities. This suggests it acts by inhibiting lipid biosynthesis and TLR4-MyD88 pathways.	
	Non-alcoholic fatty liver disease (steatosis) was modelled in HepG2 cells by treatment with palmitic acid. Scopoletin (5 µg/mL) reduced intracellular triglyceride, total cholesterol and expression of genes related to lipid metabolism (CD36, SREBP1c, PPAR-γ, and PPAR-α).	Kim HG et al. 2017
Neurological activity ***Anticholinesterase inhibition*** ***MAO inhibition*** ***Antidopaminergic*** ***Antiadrenergic***	Scopoletin inhibited monoamine oxidase (MAO) in vitro with moderate activity.	Yun et al. 2001
	Scopoletin, isolated from twigs of *Vaccinium oldhami*, inhibited acetylcholinesterase (AChE) in vitro in a dose-dependent manner (IC_{50} 10.0 µg/mL).	Lee et al. 2004
	In an in silico model for AChE inhibition, scopoletin demonstrated moderate, dose-dependent, long-lasting inhibitory activity. In rats in vivo (2 µmol intracerebro-ventricularly i.c.v.) it increased extracellular acetylcholine (ACh) concentration in rat brain to 170%.	Rollinger et al. 2004
	Scopoletin inhibited AChE by 53% and butyryl-cholinesterase (BChE) by 74% at 1 mg/mL in vitro.	Orhan et al. 2008
	In mouse models of depression, scopoletin reduced immobility time in the tail suspension test (10–100 mg/kg, p.o.), but not in the forced swimming test. It had no stimulant effect in the open field test. Using receptor antagonists, it was shown that the antidepressant-like effect of scopoletin is dependent on serotonergic (5-HT_{2A} receptors), noradrenergic (α_1- and α_2-adrenoceptors) and dopaminergic (dopamine D1 and D2 receptors) systems.	Capra et al. 2010
	Scopoletin enhanced the K+-stimulated release of ACh from rat frontal cortex synaptosomes in vitro (Emax 4 µM), an effect blocked by nicotinic ACh receptor (nAChR) antagonists, mecamylamine and dihydro-β-erythroidine. It potentiated long-term potentiation evoked by high-frequency stimulation in rat brain hippocampal slices (4 µM for 4 min), also shown to be an effect on nAChR. In vivo, scopoletin (2 µg, i.c.v.) increased T-maze alternation and ameliorated novel object recognition in mice with scopolamine-induced cholinergic deficit. It reduced age-associated deficits in object memory of 15–18-month-old mice (2 mg/kg s.c.).	Hornick et al. 2011
	Scopoletin, isolated from leaves of *Canarium patentinervium* Miq. (Burseraceae Kunth.) inhibited AChE in vitro (IC_{50} 270 µM).	Mogana et al. 2014
	Scopoletin (1–200 µg/mL), isolated from *Morinda citrifolia* Linn., dose-dependently inhibited contractility evoked by submaximal concentrations of both dopamine and noradrenaline, in isolated rat deferens. These antidopaminergic and adrenergic effects were still found at high concentrations (0.5–5 mg/mL).	Pandy et al. 2014

Biological/biochemical activity	Findings	References
	Scopoletin showed dose-dependent neuroprotective activity (at 10, 50, and 100 µM) against glutamate-induced neurotoxicity in immortalised HT22 cells from mouse hippocampus.	Lee et al. 2015
	Scopoletin (0.5, 2, 5 and 20 µg/mL), isolated from *A. annua*, inhibited AChE in vitro by 53% (IC_{50} 70 µg/mL).	Chougouo et al. 2016
	Scopoletin, isolated from *Angelica decursiva* and *Artemisia capillaris*, showed no inhibitory activity against AChE, BChE or β-site amyloid precursor protein cleaving enzyme 1 (BACE1) in vitro.	Ali et al. 2016
	Scopoletin was a potent, specific inhibitor of AChE (IC_{50} 10.2 µM). Scopoletin showed strong hydrogen bonding to several important amino acid of AChE. Hydrophobic interactions may also explain the potency to inhibit AChE.	Kuppusamy et al. 2016
	In a mouse model for scopolamine-induced amnesia, scopoletin (2.5, 5, 10 and 15 mg/kg, orally) evaluated for memory-enhancing activity. Scopoletin dose dependently attenuated the scopolamine-induced amnesic effect. At 10 and 15 mg/kg it showed activity comparable to a standard drug, donepezil. It also inhibited AChE.	Malik et al. 2016
	Scopoletin inhibited human recombinant MAO-B but had less effect on MAO-A. Docking studies explained the selectivity mechanism towards MAO isoforms. In vivo, in mice, scopoletin (80 mg/kg, i.p.) increased dopamine and decreased its metabolite DOPAC in striatum.	Basu et al. 2016
	Scopoletin (0.05, 0.1, 0.5, and 1 mg/kg bw, orally) had a dose-dependent, “U-shaped” effect on climbing and stereotyped behaviours induced by apomorphine and methamphetamine, respectively, in a mouse model for schizophrenia/psychotic behaviour. A reduction in climbing and stereotyped behaviours was observed only at 0.1 mg/kg.	Pandy & Vijeepallam 2017
	In a rat stroke models generated by occlusion of the middle cerebral artery, pre-administration of either scopoletin or scopoletin covalently bonded to lipoic acid (UPEI-400) decreased infarct volume in the 0.5 h ischaemia/5.5 h reperfusion model, but not in the 6 h occlusion model of stroke. UPEI-400 was ~1000 times more potent compared to scopoletin alone.	Connell et al. 2017
	Scopoletin inhibited AChE (IC_{50} 36 µM) but did not inhibit human recombinant monoamine oxidase (MAO-A or MAO-B) activity in vitro.	Baek et al. 2019
	Scopoletin and 5 other coumarins had high selective binding to human MAO-A in comparison with human MAO-B, but the inhibitory activity of scopoletin was the weakest compared with the other coumarins and did not suppress lipid peroxidation in rat brain homogenate.	Seong et al. 2019
	In an autoimmune encephalomyelitis (EAE) mouse model for multiple sclerosis (MS), scopoletin (50 mg/kg bw i.p.) given daily for 20 days reduced severity of the disease and decreased inflammation and demyelination of central nervous system (CNS).	Zhang et al. 2019

Biological/biochemical activity	Findings	References
	Disease alleviation correlated with downregulation of major histocompatibility complex (MHC) class II, CD80 and CD86, expressed on dendritic cells of CNS or spleens, and with infiltration and polarization of encephalitogenic Th1/Th17 cells. In vitro, scopoletin-treated, bone marrow-derived dendritic cells showed reduced expression of MHC class II, CD80 and CD86, and reduced NF-κβ phosphorylation.	
	Pre-treatment with scopoletin (30µM) prevented rotenone-induced cell death in SH-SY5Y cells, an in vitro model for Parkinson's Disease (rotenone increases oxidation). Scopoletin increased nuclear Nrf2, attributed to phosphorylation of Nrf2 by PKC-*δ*, which was also augmented on pre-treating with scopoletin. Scopoletin augments the Nrf2/ARE pathway, involved in oxidative cell death, by increasing levels of DJ-1 and preventing cytosolic degradation of Nrf2 by reducing the levels of its negative regulators Keap1 and Cullin3.	Narasimhan et al. 2019
Antidiabetic activity	In high-glucose-induced, insulin-resistant HepG2 cells in vitro, scopoletin stimulated reactivation of insulin-mediated protein kinase B (Akt/PKB) phosphorylation, an effect blocked by LY294002, a specific PI3 K inhibitor. Scopoletin was less adipogenic than rosiglitazone, as shown by the extent of lipid accumulation in differentiated adipocytes. Scopoletin increased gene expression of PPARγ2 and adipocyte-specific fatty acid binding protein. This suggests scopoletin ameliorates insulin resistance in part by upregulating PPARγ2 expression.	Zhang et al. 2010
	Scopoletin, isolated from flower buds of *Magnolia fargesii*, showed high inhibitory activity on advanced glycation end products (AGES) formation (IC_{50} 2.93 µM) and rat lens aldose reductase (IC_{50} 22.5 µM). Ex vivo, cataractogenesis of rat lenses induced by xylose was inhibited by scopoletin.	Lee et al. 2010
	Scopoletin (10 mg/kg bw, orally) attenuated increases in blood glucose in methylglyoxal (MG)-treated rats given an oral glucose tolerance test. Scopoletin activated Nrf2 by Ser40 phosphorylation, resulting in metabolism of MG into D-lactic acid and inhibition of AGEs generation, reducing accumulation of AGEs in liver. Scopoletin also increased plasma translocation of glucose transporter-2 and promoted phosphorylation of Akt caused by insulin treatment in MG-treated FL83B hepatocytes. In contrast, scopoletin effectively suppressed protein tyrosine phosphatase 1B (PTP1B) expression, thereby alleviating insulin resistance.	Chang et al. 2015
	Scopoletin showed weak (35%) inhibition of α-glucosidase in vitro.	Abdullah et al. 2016
	In a model for hepatic steatosis and inflammation using high-fat diet fed type 1 diabetic mice, scopoletin in the diet (0.01% w/w daily for 11 weeks) lowered blood glucose and HbA1c and reduced glucose intolerance.	Choi et al. 2017

Biological/biochemical activity	Findings	References
	Scopoletin, in palmitate-induced rat insulinoma 5f (RIN5f) cells in vitro. In vivo, in high-fat, high-fructose diet (HFFD)-fed rats scopoletin inhibited endoplasmic reticulum (ER) stress signaling by downregulating inositol requiring enzyme 1 (IRE1α), protein kinase like endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6α) in pancreatic β-cells. Scopoletin also restored normal ER organelle ultrastructure in vivo.	Kalpana et al. 2018
	Using the same rat model, scopoletin lowered plasma glucose, insulin, and lipids in HFFD-fed rats and increased the activities of antioxidant enzymes such as SOD, CAT, and GPx. Scopoletin also reduced TBARS, lipid hydroperoxide, and protein carbonyl levels in plasma and pancreas. It activated insulin receptor substrate 1 (IRS1), phosphatidyl inositol 3-kinase (PI3K), and Akt phosphorylation and activated AMPK and enhanced the association of IRS1-PI3K-Akt. Scopoletin can improve insulin signaling through AMPK, confirming its role as an AMPK activator.	Kalpana et al. 2019
	Scopoletin inhibited α-glucosidase and α-amylase in a dose-related manner in vitro (IC_{50} 85.12 µM and 37.36 µM, respectively). In vivo, scopoletin (10 mg/kg bw orally) supressed postprandial rise in blood glucose in both normal mice and mice with streptozotocin-induced diabetes.	Jang et al. 2018
	Scopoletin increased glucose uptake in mouse 3T3-L1 (adipocyte-like) cells in vitro and expression of plasma membrane glucose transporter type 4 (PM-GLUT4). The increase in PM-GLUT4 expression was promoted by phosphorylation of protein kinase B, activation of PI3K, and enhanced intracellular glucose uptake. Scopoletin also promoted phosphorylation of AMPK and enhanced PM-GLUT4 expression. Scopoletin-induced glucose uptake in 3T3-L1 adipocytes was inhibited by treatment with PI3K inhibitor wortmannin and AMPK inhibitor compound C.	Jang et al. 2019
Antifatigue activity	In mice given scopoletin, extracted from *Morinda elliptica* leaf, for 6 weeks at 200-400 mg of extract/kg bw, gene expression analysis suggested the enhanced performance in weight-loaded swimming time to exhaustion was due to reduction in blood lactate and blood urea nitrogen, increases in liver and muscle glycogen reserve through augmenting glucose metabolism (glucose transporter type 4 and pyruvate dehydrogenase kinase 4), lipid catabolism (acyl-coenzyme A dehydrogenases and fatty acid translocase), antioxidant (SOD 2) defence responses, electron transport (COX4I2), and energy production (PGC1α, NRF1, NRF2, cytochrome C electron transport, mitochondrial transcription factor A, UCP1, and UCP3) biomarkers.	Wan Osman et al. 2018
Antithyroid activity	In *l*-thyroxine-treated rats given scopoletin, isolated from the leaves of *Aegle marmelos*, 1 mg/kg bw per day orally for 7 days, serum thyroid hormones were reduced and glucose as well as hepatic glucose-6-phosphatase activity.	Panda & Kar 2006

Biological/biochemical activity	Findings	References
	In liver it inhibited lipid peroxidation and increased SOD and CAT. This indicates that scopoletin has the potential to inhibit thyroid function and hyperglycemia without hepatotoxicity.	
Melanin synthesis	Scopoletin (0.1-50 µM), isolated from *Cirsium setidens* Nakai (Compositae), was not toxic to B16F10 murine melanoma cells and increased melanin synthesis in a dose-dependent manner (10-100 µM). As melanin synthesis increased, scopoletin stimulated total tyrosinase activity, the rate-limiting enzyme of melanogenesis. In a cell-free system, scopoletin did not increase tyrosinase activity, so is not a direct activator of tyrosinase. Scopoletin stimulated production of microphthalmia-associated transcription factor (MITF) which controls tyrosinase expression via cAMP response element-binding protein (CREB) phosphorylation in a dose-dependent manner.	Ahn et al. 2014
	In B16F10 cells, scopoletin (20-40 µg/mL) increased protein levels of tyrosinase, tyrosinase related protein-1 (TRP-1), mRNA tyrosinase, MITF, cAMP and phosphorylation of p38 mitogen-activated protein kinase (MAPK). Scopoletin-mediated increase of intracellular melanin and tyrosinase expression were attenuated by protein kinase A (PKA) inhibitors (H-89 and KT5720), but protein kinase C (PKC) inhibitor (Ro-32-0432) had no effect and p38 MAPK inhibitor (SB203580) partially blocked scopoletin-induced intracellular melanin and tyrosinase expression. Scopoletin, synergistically with cell-permeable cAMP analogue (dibutyryl cAMP), induced tyrosinase activity and melanin content in B16F10 cells. The silencing of p38 MAPK by small interfering RNA decreased scopoletin-induced tyrosinase expression in B16F10 cells. This suggests that scopoletin may induce melanin synthesis through the cAMP/PKA pathway and partially p38 MAPK activation.	Kim DS et al. 2017
	Scopoletin increased melanin content in B16F10 murine melanoma cells and increased tyrosinase activity (10 and 25µmol/L). It also increased melanin content in zebrafish.	Heriniaina et al. 2018
Hypouricemic activity	Scopoletin (50, 100, 200 mg/kg bw i.p.) inhibited activity of xanthine oxidase (XO) in liver homogenates of hyperuricemic mice. It had relatively weak, albeit competitive-type, inhibition of XO in a commercial assay. In vivo, scopoletin (100, 200 mg/kg bw i.p.) decreased uric acid production and increased uric acid secretion in urine.	Ding et al. 2005
	Scopoletin (200 mg/kg bw orally) reduced serum uric acid concentration to normal in hyperuricemic mice, but did not affect hepatic or serum XO activity.	Zeng et al. 2017

Biological/biochemical activity	Findings	References
Antimicrobial activity **Antiparasitic activity**	Scopoletin showed antimicrobial activity against Gram positive bacteria (*Actinomyces naeslundii, Actinomyces israelii,* and *Streptococcus mutans*), Gram negative bacteria (*Prevotella intermedia, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans* previously known as *Actinobacillus actinomycetemcomitans*), and *Candida albicans*.	More et al. 2012
	Scopoletin showed no anti-malarial activity against *Plasmodium falciparum* at 10 µg/mL, or anti-fungal activity against *Candida albicans*, or anti-TB activity at 50 µg/mL	Wongsa et al. 2013
	Scopoletin, isolated from *Ruta angustifolia* leaves, did not show any significant activity against hepatitis C virus (IC_{50} >30 µg/mL).	Wahyuni et al. 2014
	Scopoletin, isolated from leaves of *Micromelum integerrimum*, showed no antimicrobial activity against *Candida albicans* and *Staphylococcus aureus* at 20 µg/mL.	Wang et al. 2014
	Scopoletin, isolated from *Canarium album Raeusch* fruits, inhibited influenza virus A (IC_{50} 22.9 µg/ml).	Yang et al. 2018
	Scopoletin, isolated from stem bark of *Trichilia gilgiana* Harms. (Meliaceae), showed high activity (IC_{50} 6.804 µg/mL) against visceral leishmaniasis parasite *Leishmania donovani* but low cytotoxicity against murine macrophage RAW 264.7 cells.	Kowa et al. 2019
Antiplatelet activity	Scopoletin had antiplatelet aggregation activity via down-regulation of thromboxane A2 production and intracellular Ca2+ mobilization, which are aggregation-inducing molecules produced in activated platelets. But, scopoletin increased both the cyclic adenosine monophosphate and cyclic guanosine monophosphate levels, which are known as intracellular Ca2+ antagonists and aggregation-inhibiting molecules. Scopoletin inhibited collagen-elevated adenosine triphosphate release, suggesting that aggregation amplification through granule secretion is inhibited by scopoletin.	Kwon et al. 2019