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Review Article

Spilanthol: occurrence, extraction, chemistry and biological activities

Alan F. Barbosa[a], Mário G. de Carvalho[a], Robert E. Smith[b,*], Armando U.O. Sabaa-Srur[a,c]

[a] Departamento de Química, Instituto de Ciências Exatas, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil
[b] Park University, Parkville, MO, USA
[c] Curso de Nutrição, Centro de Ciências da Saúde, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

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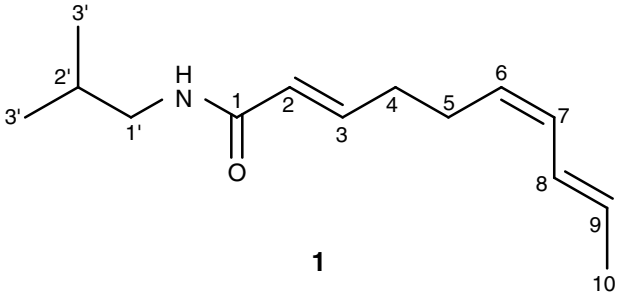
ABSTRACT

Spilanthol ($C_{14}H_{23}NO$, 221.339 g/mol) is a bioactive compound that is found in many different plants that are used as traditional remedies throughout the world. It is present in *Heliopsis longipes* and several species in the genus *Acmella*, including *A. oleracea* L., also known as paracress and jambu. Its leaves and flowers have sensory properties (pungency, tingling, numbing, mouth-watering) that make it a popular spice and ingredient in several Brazilian dishes. Spilanthol can exert a variety of biological and pharmacological effects including analgesic, neuroprotective, antioxidant, antimutagenic, anti-cancer, anti-inflammatory, antimicrobial, antilarvicidal and insecticidal activities. So, the aim of this review is to present a literature review on the spilanthol that describes its occurrence, chemistry, extraction and biological activities.

Introduction

Spilanthol ($C_{14}H_{23}NO$, 221.339 g/mol) (**1**) is a bioactive compound that is found in many different plants that are used as traditional remedies throughout the world (Molinatorres et al., 1996; Prachayasittukal et al., 2013; Paulraj et al., 2013; Rios and Olivo, 2014). Its IUPAC name is (2*E*,6*Z*,8*E*)-*N*-isobutyl-2,6,8-decatrienamide (Molinatorres et al., 1996). It is also known as affinin (Prachayasittukal et al., 2013).



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The plants in which it is found are often called toothache plants, due to the analgesic effect of spilanthol (Molinatorres et al., 1996; Hind and Biggs, 2003; Wu et al., 2008; Tiwari et al., 2011; Dias et al., 2012; Sharma et al., 2012; Abeysiri et al., 2013; Dubey et al., 2013; Prachayasittukal et al., 2013; Paulraj et al., 2013; Rios and Olivo, 2014; Dandin et al., 2014; Hajdu, 2014). Like other alkamides, it is an amphiphilic compound with a relatively polar amide and a less polar fatty acyl. So, it can be extracted from plants using either methanol, ethanol, supercritical CO_2 or hexane (Nakatani and Nagashima, 1992; Sharma et al., 2011; Dias et al., 2012; Singh and Chaturvedi, 2012a,b; Hajdu, 2014; Abeysinghe et al., 2014). After being extracted, it can be purified by preparative scale TLC and/or HPLC (Johns et al., 1982; Ogura et al., 1982; Mbeunkui et al., 2011; Pandey et al., 2011; Moreno et al., 2012; Nakatani and Nagashima, 1992; Hajdu, 2014). In addition to its oral analgesic effect, it also has antibacterial effects (Dubey et al., 2013). So, either spilanthol or extracts of plants that contain it may be added to toothpaste and used as an oral analgesic in gels (such as Buccaldol® and Indolphar®) and as an anti-wrinkle cream that can substitute for Botox in cosmetic applications (Demarne and Passaro, 2009; Veryser et al., 2014). There are also some anti-aging products (Gatuline®, SYN®-COLL, ChroNOline™) that contain spilanthol. There are about 30 patents that describe products that are made from a variety of *Splianthes* species (Haw and Keng, 2003). It is also eaten in foods. The leaves of some of the plants (like *S. acmella*) that contain spilanthol are used as a spice (Haw and Keng, 2003; Paulraj et al., 2013). The European Union estimated that the average daily intake of spilanthol was 24 μg/person/day (Veryser et al., 2014). It is also possible that spilanthol, like other alkamides, can have important effects on the central nervous system (CNS) and immune system (Gertsch, 2008; Hajdu, 2014; Veryser et al., 2014). However, its greatest potential for saving lives and improving human health may be its ability to kill mosquitoes that can spread tropical diseases like malaria and dengue fever (Pandey

* Corresponding author.
E-mail: robert.smith05@park.edu (R.E. Smith).

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et al., 2011; Spelman et al., 2011; Hernández-Morales et al., 2015). Moreover, it has anti-cancer activity (Soares et al., 2014; Mishra et al., 2015). So, the purposes of this review are to tell where spilanthol (**1**) can be found in nature, tell how it can be extracted, describe its chemistry and review its diverse health effects.

Occurrence

Spilanthol (or affinin) (**1**) can be found in not just *Acmella oleracea*, but also *A. ciliate*, *A. oppositifolia*, *A. radicans*, *A. brachyglossa*, *A. ciliate*, *A. oleracea*, *A. paniculata*, *A. uliginosa*, *Welelia parviceps* and *Heliopsis longipes* (Chung et al., 2008; Prachayasittukal et al., 2013). Many of the articles that describe its presence in *H. longipes* call it affinin instead of spilanthol (Johns et al., 1982; Rios et al., 2007; Spelman et al., 2011; Déciga-Campos et al., 2012). On the other hand, there is some disagreement in the literature over the name of the genus and species of one of the most important plants that is said to contain spilanthol. Some call it *A. oleracea* (Moreno et al., 2012; Simas et al., 2013; Abeysinghe et al., 2014; Castro et al., 2014), but others call it *A. oleracea* (L.) R. K. Jansen (Simas et al., 2013; Soares et al., 2014; de Alcantara et al., 2014), *A. oleracea* Compositae (Hind and Biggs, 2003), *S. oleracea* L. (Martins et al., 2012), *S. acmella*, (Chung et al., 2008; Demarne and Passaro, 2009; Mbeunkui et al., 2011; Pandey et al., 2011; Prachayasittukal et al., 2013; Sana et al., 2014; Soares et al., 2014; Mishra et al., 2015), *S. acmella* L. var. *oleracea* Clarke (Nakatani and Nagashima, 1992) and *S. acmella* Murr. (Asteraceae) (Singh and Chaturvedi, 2012a,b; Abeysiri et al., 2013). At least one article stated that the flower head of *S. acmella* L. var. *oleracea* Clarke are yellow, but those of *S. acmella* are purple (Nakatani and Nagashima, 1992). To add to the confusion, one review article on the genus *Spilanthes* Jacq stated that "The genus is often confused with the genus *Acmella* Rich. Ex Pers.", "Spilanthes species have discoid heads and *Acmella* species have rayed heads", and "Spilanthes has a chromosome number of 16, whereas *Acmella* has 12 or 13" (Paulraj et al., 2013). In complete contrast, another author reported that the inflorescences of *Acmella oleracea* (L.) R.K. Jansen have discoid heads and a chromosome number of 2n = 68 or 70 (Grubben and Denton, 2004). Monographs have been written about each genus (*Acmella* and *Spilanthes*) (Jansen, 1981, 1985), but the "toothache plant" was placed in the *Acmella* genus (Jansen, 1985). Some of its common names include jambu, agrião do Pará and paracress (Jansen, 1985). The monograph on *Acmella* warned of false synonyms for *A. oleracea* that appear on various websites. Some of them state that the "accepted scientific name" is *Spilanthes acmella* (L.) Murr., but the photos on them clearly show *A. oleracea* (Jansen, 1985). This monograph also stated that the "currently accepted name" for *Spilantes acmella* (L.) Murr. is *Blainvillea acmella* (L.) Philipson (Jansen, 1985). There is another article that talks about a Mexican plant that they called *Acmella* (*Spilanthes*) *oppositifolia*, while the Nahuatl name was chilcuage (Molinatorres et al., 1996). There are also five different species of *Acmella* in Taiwan that contain spilanthol (Chung et al., 2008). Finally, there is an article that lists *S. acmella* and *S. oleraceae* as being two separate plants (Tiwari et al., 2011). Other synonyms include *A. ciliata* Kunth, *Cotula pyretharia* L., *S. fusca* Mart, *Bidens fervida* Lan and *A. uliginosa* (Sw.) Cass (Borges, 2009; Costa et al., 2013).

Extraction, purification and quantitation

Since spilanthol (**1**) is amphiphilic, it can be extracted from plants using solvents that range in polarity from hexane (Ramsewak et al., 1999) to methanol:H_2O (4:1, v/v) (Abeysinghe et al., 2014). There is also an ethanolic extract that is sold in pharmacies (Boonen et al., 2010a,b). However, to the best of our knowledge no attempt has been made to compare the amount of spilanthol that can be extracted using different methods. Moreover, nobody has ever tried using pressurized liquid extraction with dry methanol, which has been shown to be able to solubilize more material from many fruits and vegetables than other methods, including Soxhlet extraction or ultrasonication (Richter et al., 1996; Richards et al., 2014; Levine et al., 2015). However, some of the previous publications do tell how much material was solubilized. For example, hexane at an unspecified temperature was able to solubilize 10 g of material from 1130 g of lyophilized flowers (Ramsewak et al., 1999). Others used ultrasonication with 60 ml of ethanol:hexane (3:7, v/v) at 50 °C and 30 min to solubilize an unspecified amount of material from 2 g of dried flowers Costa et al., 2013). Another group used an unknown amount of ethanol at room temperature to solubilize 106 g (13%) of material from 803 g of dried leaves (Simas et al., 2013). Others solubilized 15 g from 300 g of flowers using methanol at room temperature (Mbeunkui et al., 2011). Another group used methanol to solubilize 18.0, 16.6 and 10.2% of the material from dry leaves, stems and flowers, respectively (Abeysiri et al., 2013). Still others used 2.5 l of ethanol:water (7:3, v/v) to solubilize an unknown amount of material from 426 g of dried flowers (Martins et al., 2012).

Supercritical CO_2 with added ethanol and water was also used to try to extract spilanthol from *S. acmella* flowers, leaves and stems (Dias et al., 2012). It was purified from an ethanolic extract using TLC using silica gel plates and hexane:ethyl acetate (2:1, v/v) as the mobile phase (Dias et al., 2012). TLC was also used to purify spilanthol from dry *A. oleracea* flowers that was first extracted with ultrasonication and ethanol:hexane (3:7, v/v) at 50 °C and 30 min (Costa et al., 2013). Others used TLC followed by preparative scale HPLC to purify spilanthol from hexane extracts of flowers (Nakatani and Nagashima, 1992). Another group used two preparative scale columns (XAD-16 and Sephadex LH-20) followed by preparative scale TLC to purify spilanthol from leaves (Simas et al., 2013). Another approach that proved successful was column chromatography on silica gel, followed by TLC (Ramsewak et al., 1999). Finally, centrifugal partition chromatography using a mixture of heptane, ethyl acetate, methanol and water (3:2:3:2, v/v) was used to purify spilanthol (Mbeunkui et al., 2011).

For quantitation, both HPLC with UV detection and LC–MS have been used (Bae et al., 2010; Sharma et al., 2011; Singh and Chaturvedi, 2012a,b). Both methods used a C18 column for the separation. One HPLC method used an isocratic mobile phase consisting of 93:7 CH_3CN:H_2O (v/v), flowing at 0.5 ml/min (Singh and Chaturvedi, 2012a,b). The retention time for spilanthol was 7.34 min (Prachayasittukal et al., 2013). Another HPLC method used isocratic elution with CH_3CN:H_2O (1:1, v/v) flowing at 0.2 min (Bae et al., 2010). The retention time was 4.97 min (Bae et al., 2010). One LC–MS method used a gradient elution that started with 1:4 CH_3CN:H_2O (v/v), containing 1% acetic acid and increased to 9:1 CH_3CN:H_2O (v/v) over 150 min (Sharma et al., 2011). The retention time of spilanthol was 62.37 min (Sharma et al., 2011). The other LC–MS method was validated for quantifying spilanthol in a mixture of unspecified amounts of leaves, flower buds and roots, which were extracted with ethanol:water (19:1, v/v) at room temperature (Bae et al., 2010). The combined peak areas due to the $[M+H]^+$ and $[2M+H]^+$ ions with *m/z* of 222 and 443 were used for quantitation (Bae et al., 2010). In addition, fragment ions with *m/z* of 123, 81, 121, 67 and 149 were also seen. However, the method was validated by simply analyzing spilanthol standards dissolved in an unspecified solvent, showing that a linear calibration curve could be obtained and by testing the repeatability of the analysis of standards. Recoveries of spilanthol that were added to the samples (spiked samples) were not measured. It is also quite likely that the method was not used to actually quantify spilanthol in any samples. There is a table that showed the spilanthol concentrations that were found in extracts of the plant that they called *S. acmella* but

Table 1
^{1}H and ^{13}C NMR chemical shifts (ppm) of spilanthol (**1**) in $CDCl_3$ (Nakatani and Nagashima, 1992).

H no.	δ ^{1}H (ppm)	C no.	δ ^{13}C (ppm)
H-2	5.79 br; d	C-1	166.0
3	6.83 dt	2	124.2
4	2.23–2.35 m	3	143.5
5	2.23–2.35 m	4	32.1
6	5.26 dt	5	26.4
7	5.97 dd	6	127.7
8	6.29 br; dd	7	129.5
9	5.70 dq	8	126.7
10	1.78 d	9	130.0
H-N	5.47 br, s	10	18.3
1′	3.15 dd	1′	46.9
2′		2′	28.6
3′	1.78 m	3′	20.1

the results were expressed as mg/ml, as if they were concentrations of standards dissolved in solvents. There was no mention of concentrations of spilanthol in units of μg spilanthol per mg of sample (Bae et al., 2010). However, a method based on HPLC with UV detection at 237 nm was used to find 3294 μg/g spilanthol per dry weight in the leaves of *in vitro* plants and 2704 μg/g dry leaves in the leaves of *in vivo* plants (Singh and Chaturvedi, 2012a,b). However, no attempt was made to compare the amount of spilanthol that could be extracted using pressurized liquid extraction, sonication or Soxhlet extraction. It is also quite likely that the concentration of spilanthol is different in different parts of the plant. So, there is clearly a need for an analysis of different parts of genuine *A. oleracea*.

Chemistry

Spilanthol (**1**) is an *N*-alkylamide, many of which have various bioactivities, from helping to protect plants to being an antibacterial, antifungal, analgesic and endocannabinoid agonists (Veryser et al., 2014). One article reported that there over 200 alkamides have been found in ten families: Aristolochiaceae, Asteraceae, Brassicaceae, Convolvulaceae, Euphorbiacea, Menispermaceae, Piperaceae, Poaceae, Rutaceae and Solanaceae (Molina-Torres et al., 2004). Another group reported that over 400 *N*-alkylamides have been identified in 26 different plant families (Gertsch, 2008). There is also an alkamide database that has more details in it (Boonen et al., 2012).

The stereoselective synthesis of spilanthol with a 61% yield has been reported (Ikeda et al., 1984). It is light yellow with a melting point of 23 °C, a boiling point of 165 °C, a refractive index at 298 °C of 1.5135 and a maximum UV absorption at 228.5 nm (Jacobson, 1957). Its IR spectrum was reported as having the following major peaks: ν_{max} (film) cm^{-1}: 3340, 3150, 3080, 3020, 1678, 1636, 1550, 1240, 1160, 987, 953 (Nakatani and Nagashima, 1992). It has a monoisotopic molecular weight of 221.177963 Da. So, the positive ion mass spectrum contains a molecular ion $[M+H]^+$ $m/z = 222$ and a fragment $[MH-C_4H_{11}N]^+$ with $m/z = 149$ (loss of isobutyl amine group) as well as a fragment with $m/z = 99$, that showed the presence of an isobutylamide (Jacobson, 1957). Its ^{1}H and ^{13}C NMR spectra have been reported (Nakatani and Nagashima, 1992). Chemical shifts are listed in Table 1.

The parts of spilanthol that are important for its analgesic activity, tingling and mouth-watering effects (pharmacophores) are the amide and unsaturated (alkenyl) fatty acyl (Ley et al., 2006; Rios and Olivo, 2014).

Biological activities

Spilanthol has many biological activities (Dubey et al., 2013), including analgesic (Molinatorres et al., 1996; Hind and Biggs, 2003; Wu et al., 2008; Cilia-López et al., 2010; Tiwari et al., 2011; Dias et al., 2012; Sharma et al., 2012; Abeysiri et al., 2013; Dubey et al., 2013; Prachayasittukal et al., 2013; Paulraj et al., 2013; Rios and Olivo, 2014; Dandin et al., 2014; Hajdu, 2014), antinociceptive (Rios et al., 2007; Déciga-Campos et al., 2012), antioxidant (Abeysiri et al., 2013), anti-inflammatory (Wu et al., 2008; Hernández et al., 2009; Dias et al., 2012), antimutagenic (Arriaga-Alba et al., 2013), anti-wrinkle (Demarne and Passaro, 2009), antifungal (Dubey et al., 2013), bacteriostatic (Molina-Torres et al., 2004), insecticidal (Kadir et al., 1989; Sharma et al., 2012; Moreno et al., 2012), anti-malarial (Sharma et al., 2012), anti-larvicidal activities against *Aedes aegypti* and *Helicoverpa zea* neonates (Ramsewak et al., 1999), and anti-molluscicidal activities (Johns et al., 1982). There have also been reports on its activities as an anticonvulsant, antioxidant, aphrodisiac, pancreatic lipase inhibitor, antimicrobial agent, antinociceptive agent, diuretic, vasorelaxant, anti-human immunodeficiency virus, toothache relief and as an anti-inflammatory agent (Dubey et al., 2013). It can be absorbed through the skin, endothelial gut, oral mucosa and blood–brain barrier (Boonen et al., 2010a,b; Veryser et al., 2014). It can enhance the ability of caffeine, fortestosterone and five mycotoxins to penetrate the skin (De Spiegeleer et al., 2013). So, it is important to make sure that formulations containing spilanthol are not contaminated with mycotoxins (De Spiegeleer et al., 2013). It also improved male sexual performance in rats as indicated by penile erection, mounting frequency, intromission frequency, ejaculation frequency that lasted even 14 days after discontinuing its administration (Sharma et al., 2011).

The antinociceptive activity of spilanthol was studied in detail (Déciga-Campos et al., 2010). Intraperitoneal administration of 30 mg/kg spilanthol produced an antinociceptive dependent-dose effect when assessed in mice submitted to acetic acid and capsaicin tests. Spilanthol-induced antinociception was blocked by naltrexone, *p*-chlorophenylalanine and flumazenil. So, its antinociceptive effect may be due to the activation of opioidergic, serotoninergic and GABAergic systems. Moreover, the antinociceptive effect decreased when mice were pretreated with 1*H*-[1,2,4]oxadiazolo[1,2-*a*]quinoxalin-1-one and glibenclamide. This supports the idea that the nitric oxide-K^+ channels pathway could be involved in the mechanism of action (Déciga-Campos et al., 2010). Subsequently, the same group found that spilanthol not only had a antinociceptive effect, but it also modified anxiety behavior and prolonged the time of sodium pentobarbital-induced hypnosis. They also found that spilanthol decreased the time of clonic and tonic seizures that were induced by pentylenetetrazole (PTZ) (Déciga-Campos et al., 2012).

Analgesic activity was studied by evaluating the inhibition of acetic acid induced writhing in mice (Ogura et al., 1982). Spilanthol was administered orally in aqueous solutions at doses ranging from 2.5 to 10.0 mg/kg. It exhibited an ED_{50} of 6.98 mg/kg. The analgesic activity of spilanthol was attributed to increased GABA release in the temporal cerebral cortex (Ogura et al., 1982). In another study, spilanthol caused GABA to be released 0.5 min after being administered at a concentration of 1×10^{-4} M. One other study found that spilanthol displayed analgesic action similar to ketorolac (Cilia-López et al., 2010). Also, its stimulating effect on the nervous system of adult mice was comparable to caffeine (Cilia-López et al., 2010).

The antimutagenic activity of spilanthol was demonstrated by its ability to reduce 2AA- and NOR-induced mutations inTA98 and TA102 strains of *Salmonella Typhimurium* (Arriaga-Alba et al., 2013). Spilanthol (25 and 50 μg/plate) significantly reduced the frameshift mutations that were generated by 2-aminoanthracene (2AA) (40%) and reduced the oxidative DNA damage generated by norfloxacin (NOR) (37–50%) (Arriaga-Alba et al., 2013).

The antioxidant power of spilanthol and extracts of *A. oleracea* have also been studied (Abeysiri et al., 2013). One study found 5.29,

1.42 and 3.42 mg of trolox equivalents per g of dry leaves, stems and flowers (Abeysiri et al., 2013). It also found 7.59, 1.65 and 5.34 mg of gallic acid equivalents per gram dry weight (mg GAE/g DW) of total phenolic compounds (Abeysiri et al., 2013). A different study found 9.2, 10.3 and 7.7 mg of trolox equivalents per g of dry arial parts of *A. oleracea* grown three different ways: in the field, with hydroponics and as a callus, respectively (Abeysinghe et al., 2014). The same study found 11.0, 11.5 and 9.9 mg GAE/g DW total phenolics in *A. oleracea* grown in the field, with hydroponics and as a callus, respectively (Abeysinghe et al., 2014). The total flavonoid content was 11.3, 12.3 and 7.4 mg rutin equivalents per gram of dry weight in *A. oleracea* grown in the field, with hydroponics and as a callus, respectively.

The anti-inflammatory activity of dried flowers was demonstrated on the commonly used lipopolysaccharide-activated murine macrophage model, RAW 264.7 (Wu et al., 2008). These macrophages produce nitric oxide (NO) to mediate inflammation, through an inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2). Spilanthol inhibited the production of iNOS and COX-2 and the mRNA that code for them. It was also suggested that spilanthol attenuates the inflammatory responses in murine RAW 264.7 macrophages partly due to the inactivation of NF-κB. This down regulates the production of proinflammatory mediators. Spilanthol also had an anti-inflammatory effect on the arachidonic acid model with ED_{50} = 1.2 mg/ear (Wu et al., 2008). In a different study using the phorbol myristate acetate model, spilanthol showed an anti-inflammatory dose-dependent effect with ED_{50} = 1.3 mg/ear (Hernández et al., 2009).

Extracts containing spilanthol have been used to treat toothaches, stomatitis and skin diseases such as swimmer's eczema (Boonen et al., 2010a,b). Extracts and spilanthol are in buccal mucosa preparations that are indicated for a painful mouth and minor mouth ulcers. Several spilanthol containing preparations for buccal use are commercially available (Boonen et al., 2010a,b). Also, spilanthol has been incorporated in tooth pastes and mouth rinses. The objective is to provide a lasting fresh minty flavor; it also increases salivation, which improves appetite. The spilanthol present also has a mild anesthetic effect thus enabling people with toothache to brush comfortably (Hatasa and Iioka, 1973). There is also a patent for manufacturing toothpastes or other oral compositions with spilanthol-rich essential oils (Shimada and Gomi, 1995). A mouthwash contained ethanol 10.0, 85% glycerin 8.0, 65% sorbitol 2.0, chlorohexidine gluconate 0.05, triclosan 0.003, menthol 0.01, peppermint oil 0.01, sodium saccharin 0.001, spilanthol-rich essential oil 0.01 wt.% and balance purified water (Shimada and Gomi, 1995).

Also, spilanthol in *A. oleracea* L. extracts inhibited contractions in subcutaneous muscles, notably those of the face, and can be used as an anti-wrinkle product (Demarne and Passaro, 2009). As a result, many anti-aging products containing spilanthol such as Gatuline®, SYN®-COLL and ChroNOline™ are available.

The antifungal and bacteriostatic activities of spilanthol and other alkamides from the roots of *H. longipes* were also studied (Molina-Torres et al., 2004). Four of the assayed fungi showed growth inhibition of 100% due to the presence of spilanthol: *Sclerotium rolfsii*, *S. cepivorum*, *Phytophthora infestans*, and *Rhizoctonia solani* AG-3 and AG-5. Spilanthol also inhibited the growth of *Bacillus subtilis*, *Escherichia coli* and *Saccharomyces cerevisiae* at concentrations as low as 25 μg/ml (Molina-Torres et al., 2004). In another study, spilanthol in *S. calva* was found to have antifungal activity against the fungi *Fusarium oxysporum* and *Trichophyton mentagrophytes* (Rai et al., 2004). This antifungal activity was enhanced when *S. calva* was inoculated with the root endophyte *Piriformospora indica*, which also increased the concentration of spilanthol in the roots of *S. calva* (Rai et al., 2004).

Spilanthol was also shown to be useful as an insecticide (Kadir et al., 1989; Spelman et al., 2011; Sharma et al., 2012). It killed the diamondback moth, *Plutella xylostella* L, which is one of the most destructive pests that attack cruciferous vegetables, such as broccoli (Sharma et al., 2012). Spilanthol was also able to kill the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which attacks solanaceous plants and has become a serious threat to tomatoes in the Mediterranean region (Moreno et al., 2012). Electrophysiological studies indicated immediate hyperexcitation followed by complete inhibition of the cockroach cercal nerve activity. Spilanthol exhibited the highest toxicity to *Tuta absoluta*, with the lowest LD_{50} (0.13 μg mg^{-1}). Furthermore, spilanthol was approximately five times more toxic than permethrin and approximately 321 times more potent than *Azadirachta indica* extract. On the other hand, spilanthol was not toxic to two beneficial insects, the predator *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) and the pollinator, *tetragonisca angustula* (Latr.) (Hymenoptera: Apidae: Melipninae) (Moreno et al., 2012). Even more important, spilanthol has been shown to be toxic to the mosquitoes (*Plasmodium falciparum*) that carry malaria (Spelman et al., 2011). It had an IC_{50} of 16.5 μg/ml and 41.4 μg/ml on *P. falciparum* strain PFB and IC_{50} of 5.8 μg/ml and 16.3 μg/ml for the chloroquine resistant *P. falciparum* K1 strain, respectively. Further investigations revealed that at relatively low concentrations, spilanthol and the water extract of *S. acmella* reduced the parasitemia 59 and 53% in mice infected with *P. yoelii yoelii* 17XNL at 5 and 50 mg/kg, respectively. This parasite is used to infect mice in an animal model of malaria. These results provide evidence supporting the antimalarial activities of *S. acmella* and spilanthol (Spelman et al., 2011). Finally, another group reported the ability of extracts of *S. acmella* Murr. to kill the American cockroach, *Periplaneta americana* L. (Kadir et al., 1989). The potency was found to be 1.3, 2.6 and 3.8 times more toxic than carbaryl, bioresmethrin and lindane, respectively (Kadir et al., 1989).

Spilanthol is also active against *Aedes aegyptii* larvae, which can spread the viruses that cause dengue fever, chikungunya, and yellow fever as well as *Helicoverpa zea* neonates (corn earworm) at concentrations of 12.5 and 250 mg/ml, respectively (Ramsewak et al., 1999). Spilanthol, at 7.5 ppm concentration, caused 100% motility of eggs, larvae, and pupae of *Anopheles*, *Culex*, and *Aedes* mosquitoes at lower doses; it is also effective against eggs and pupae (Saraf and Dixit, 2002). The insecticidal activity of *Heliopsis longipes* roots against *Anopheles albimanus* and *Aedes aegypti* was determined (Hernández-Morales et al., 2015). A concentration of 7 mg/l of ethanolic extract caused 100% of larval mortality for *A. albimanus*, and had the same effect on *A. aegypti* larvae. This effect could be attributed to spilanthol. The conjugated double bonds present in its structure were found to be necessary to maintain larvicidal activity. This study demonstrated the potential of *H. longipes* for controlling the larval stage of *A. albimanus* and *A. aegypti*, transmitter vectors of malaria and dengue fever, respectively (Hernández-Morales et al., 2015).

Others explored *Spilanthes acmella* Murr. for insecticidal activity (Sharma et al., 2012). The seed extract and spilanthol were toxic to *Plutella xylostella*. An activity of 95–100% was observed at a dose of 2 g/l of spilanthol, while 60–70 and 80–90% mortality was seen in crude seed extracts prepared in methanol and hexane at a dose of 5 g/l after 48 h exposure. LC_{50} values of 1.49, 5.14, 5.04, 11.75 g/l were observed for spilanthol, crude methanolic seed extract, hexane extracts and deltamethrin, respectively. These findings indicated the potential of *S. acmella* and spilanthol for controlling *P. xylostella* and other insects of agricultural importance (Sharma et al., 2012). Spilanthol also has strong molluscicidal activity against *Physa occidentalis* (LD_{50} of 100 μM) and the cercariae of the fluke (Johns et al., 1982). At a concentration of 50 mg/l in water at 21° snails were inactive after 60 min and dead within 18 h. At

150 mg/l (the solubility limit for spilanthol) cercarial emergence ceased and the snails showed immobility after 30 min. Cercariae ceased to move after five set and convulsed after 1 min (Johns et al., 1982).

Spilanthol also can also stimulate the growth of roots in *Arabidopsis thaliana* seedlings (Campos-Cuevas et al., 2008). Although the effects of spilanthol was similar to those produced by auxins on adventitious root development, the ability of shoot explants to respond to spilanthol was found to be independent of auxin signaling. These results suggest a role for spilanthol in regulating adventitious root development, probably operating through the NO signal transduction pathway (Campos-Cuevas et al., 2008).

Spilanthol was also shown to inhibit CYP P450 enzymes, with IC_{50} values of 25, 16.1 and 13.5 μg/ml for CYP1A1/2, CYP2D6 and CYP3A4, respectively (Rodeiro et al., 2009). These results suggest that spilanthol inhibits the major human P450 enzymes involved in drug metabolism and could induce potential herbal–drug interactions (Smith, 2014). On the other hand, CYP1A1/2 inhibition could be associated with decreased carcinogenic risk. Although, *in vitro* inhibition of P450s does not necessarily lead to relevant *in vivo* effects, these results recommend a cautious evaluation of the potential clinical consequences derived from the consumption of these products, particularly for long-term treatments (Rodeiro et al., 2009).

In conclusion, spilanthol is a secondary metabolite with high industrial potential as well as several biological properties and health effects. It can be found, extracted and purified from *A. oleracea* and *H. longipes*. *A. oleracea* is used as a spice and a food in the northern part of Brazil. It is also used as a treatment for treating toothaches, so it is called the toothache plant. Spilanthol may also have analgesic (Molinatorres et al., 1996; Hind and Biggs, 2003; Cilia-López et al., 2010; Tiwari et al., 2011; Dias et al., 2012; Sharma et al., 2012; Dubey et al., 2013; Prachayasittukal et al., 2013; Paulraj et al., 2013; Wu et al., 2008; Rios and Olivo, 2014; Dandin et al., 2014; Hajdu, 2014), antinociceptive (Rios et al., 2007; Déciga-Campos et al., 2012), antioxidant (Abeysiri et al., 2013), anti-inflammatory (Wu et al., 2008; Hernández et al., 2009; Dias et al., 2012), antimutagenic (Arriaga-Alba et al., 2013), anti-wrinkle (Demarne and Passaro, 2009), antifungal (Dubey et al., 2013), bacteriostatic (Molina-Torres et al., 2004), insecticidal (Kadir et al., 1989; Sharma et al., 2012; Moreno et al., 2012), anti-malarial (Soares et al., 2014), anti-larvicidal against *Aedes aegypti* and *Helicoverpa zea* neonates (Ramsewak et al., 1999), and anti-molluscicidal (Johns et al., 1982). There have also been reports on its activities as an anticonvulsant, antioxidant, aphrodisiac, pancreatic lipase inhibitor, antimicrobial agent, antinociceptive agent, diuretic, vasorelaxant, anti-human immunodeficiency virus, toothache relief and anti-inflammatory (Dubey et al., 2013). The biological activities are listed in Box 1.

However, the human toxicity of spilanthol has not been thoroughly tested, even though *A. oleracea* and *H. longipes* have been consumed for a long time. Also, the concentrations of spilanthol in different parts of these plants have not been determined.

Box 1
Biological activities of spilanthol.

Biological activity	Reference
Analgesic	Prachayasittukal et al. (2013)
Antinociceptive	Déciga-Campos et al. (2012)
Antioxidant	Abeysiri et al. (2013)
Anti-inflammatory	Dias et al. (2012)
Anti-wrinkle	Demarne and Passaro (2009)
Antifungal	Dubey et al. (2013)
Bacteriostatic	Molina-Torres et al. (2004)
Insecticidal	Sharma et al. (2012)
Antimalarial	Sharma et al. (2012)
Anti-larvicidal against *Aedes aegypti* and *Helicoverpa zea* neonates	Ramsewak et al. (1999)
Anti-molluscicidal	Johns et al. (1982)
Anticonvulsant	Dubey et al. (2013)
Aphrodisiac	Dubey et al. (2013)
Pancreatic lipase inhibitor	Dubey et al. (2013)
Antimicrobial agent	Dubey et al. (2013)
Diuretic	Dubey et al. (2013)
Vasorelaxant	Dubey et al. (2013)
Anti-human immunodeficiency virus	Dubey et al. (2013)
Toothache relief	Dubey et al. (2013)
Enhance skin penetration of caffeine, fortestosterone and five mycotoxins	Dubey et al. (2013)

Authors' contributions

AFB, MGC, RES and AUOSR all contributed to the concept, literature search and writing of this review article.

Conflicts of interest

The authors declare no conflicts of interest.

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