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Review article:

HIGH THERAPEUTIC POTENTIAL OF *SPILANTHES ACMELLA*: A REVIEW

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ABSTRACT

Spilanthes acmella, a well known antitoothache plant with high medicinal usages, has been recognized as an important medicinal plant and has an increasingly high demand worldwide. From its traditional uses in health care and food, extensive phytochemical studies have been reported. This review provides an overview and general description of the plant species, bioactive metabolites and important pharmacological activities including the preparation, purification and *in vitro* large-scale production. Structure-activity relationships of the bioactive compounds have been discussed. Considering data from the literature, it could be demonstrated that *S. acmella* possesses diverse bioactive properties and immense utilization in medicine, health care, cosmetics and as health supplements. As a health food, it is enriched with high therapeutic value with high potential for further development.

Keywords: *Spilanthes acmella*, bioactive metabolites, bioactivities, structure-activity relationships, *in vitro* production

INTRODUCTION

Plants contain a diverse group of highly valuable and readily available resource of bioactive metabolites, e.g. alkaloids, tannins, essential oils and flavonoids (Jagan Rao et al., 2012; Prachayasittikul et al., 2008, 2009b 2010a), which have been used in medicinal practices for a long time (Tiwari et al., 2011). Thus far, medicinal plant is an alternative medicine (Consult, 2003) that is still in use and is a popular choice for primary health care(Vanamala et al., 2012). However, if improperly used plants can also be toxic (Perry et al., 2000). The World Health Organization has estimated that about 80 % of the population in developing countries are unable to afford drugs and rely on traditional medicines especially those that are plant-based (Elumalai et al., 2012) such as India (Jain et al., 2006; Little, 2004), Sri Lanka (Ediriweera 2007), Bangladesh (Rahmatullah et al., 2010), China and Japan (Little, 2004) including Thailand (Phongpaichit et al., 2005; Sawangjaroen et al., 2006).

The practice of botanical healing slowly disappeared from western countries with the introduction and advent of science and technology (Tyagi and Delanty, 2003).

However, the uses of traditional medicine dramatically increased in Europe and North America in the last 50 years (Tyagi and Delanty, 2003).

Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), antirheumatics (62 %) (Jain et al., 2006), diuretics (60.22 %) (Kumar et al., 2010; Vanamala et al., 2012), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, antiobesity and antihypertention. In dental care it has been employed as anticariogenic (Ferrazzano et al., 2011), analgesic (Abascal and Yarnell, 2010; Kroll, 1995), local anesthetic (Abascal and Yarnell, 2010), wound healing agents (Abascal and Yarnell, 2001, 2010; Jagan Rao et al., 2012), antiinflammation (Abascal and Yarnell 2001, 2010) and recurrent aphthous stomatitis treatment (Abascal and Yarnell 2010). It has also been used for beauty care (Artaria et al., 2011; Demarne and Passaro, 2009) and as health food e.g. curcumin (*Curcuma longa* Linn.) (Kohli et al., 2005), ginger (*Zingiber officinale*) (Kubra and Rao, 2011), lemon grass (*Cymbopogon citrates* Stapf) (Nanasombat and Teckchuen, 2009), green shallot (*Allium cepa var. aggregatum*) (Rabinowitch and Kamenetsky, 2002), garlic (*Allium sativum* L.) (Borek, 2010), holy basil (*Ocimum sanctum* Linn.) (Singh et al., 1996), sweet basil (*Ocimum basilicum* L.) (Lee et al., 2005), hairy basil (*Ocimum basilicum* L.f. var. citratum Back.) (Chanwitheesuk et al., 2005) and kitchen mint (*Mentha cordifolia* Opiz.) (Özbek and Dadali, 2007).

Recently, health foods, herbs as well as dietary supplements enriched with medicinal ingredients such as antioxidants and bioactive metabolites have drawn considerable attention worldwide, especially herbs that are used as food and traditional medicine (Tyagi and Delanty, 2003). Our concern centers around medicinal plants bearing bioactive compounds, which are employed as therapeutics and health care (Abascal and Yarnell, 2010). Therefore, *Spilanthes acmella* Murr. is a plant of great interest owing to its known reputation as an antitoothache plant and hold tremendous medicinal usages. This review focuses on the general background, therapeutic uses, bioactive compounds and large-scale production.

General

Spilanthes (Compositae or Asteraceae) is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia (Sahu et al., 2011; Tiwari et al., 2011). *S. acmella* is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 centimeters tall. It is grown in damp area (Tiwari et al., 2011; Wongsawatkul et al., 2008) and has low rate of germination or poor vegetative propagation (Tiwari et al., 2011). Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness (Wongsawatkul et al., 2008). The plant species has been used commonly as a folk remedy, e.g. for toothache, rheumatic and fever (Wongsawatkul et al., 2008), as fresh vegetable (Tiwari et al., 2011) as well as spice for Japanese appetizer (Leng et al., 2011).

Traditional uses

The whole plants (e.g. flowers, leaves, roots, stems and aerial parts) of *Spilanthes* have been used in health care (Leng et al., 2004; Ospina De Nigrinis et al., 1986; Purabi and Kalita, 2005; Research, 1976; Rios-Chavez et al., 2003; Senthilkumar et al., 2007; Tiwari and Kakkar, 1990) and food (Barman et al., 2009; Boonen et al., 2010; Wu et al., 2008).Particularly, *S. acmella* or *S. oleracea* (paracress or eyeball plant), is a well-known antitoothache plant (Sahu et al., 2011) and has been used as traditional medicine for many purposes

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(Table 1). So far, various Thai medicinal plants have been used for the remedy of toothache as well as used in dental applications (Table 2).

Bioactive metabolites

Extensive phytochemical investigations of *S. acmella* had previously been reported. It constitutes a diverse group of compounds. Major isolates were lipophilic alkylamides or alkamides bearing different number of unsaturated hydrocarbons (alkenes and alkynes), such as spilanthol (**1**) or affinin *(2E,6Z,8E)-N*-isobutyl-2,6,8-decatrienamide (Gokhale and Bhide, 1945; Ramsewak et al., 1999) and amide derivatives **2**–**8** (Figure 1). In general, when alkamides are chewed, a pungent taste is released and causes itch and salivation (Rios, 2012). Alkamides are structurally related to animal endocannabinoids and is highly active in the central nervous system. Particularly, anandamide (*N*-arachidonoyl-ethanolamine, **9**) is an endogenous cannabinoid cerebral neurotransmitter (Figure 1).

Spilanthol was first isolated in 1945 from the flower head ethanol (EtOH) extract of *S. acmella*. In early 1903, it was first obtained from the different plant species, *S. acmella* L.var. *oleracea* Clarke (Gokhale and Bhide, 1945). Aside from being found in *S. acmella*, spilanthol was also found in other plant species as shown in Table 3 (Rios, 2012).

The synthesis of spilanthol was reported in multistep and afforded low overall yields. However, an efficient synthetic method had been developed (Wang et al., 1998). Thus far, the spilanthol is commercially available in form of alcoholic (65 % EtOH) extract or A. Vogel *Spilanthes*.

Table 1: Traditional uses and applications of *S. acmella*

Health care	Treatment	Plant extract	References
Medical	Rheumatism, fever Diuretics Flu, cough, rabies diseases, Tuberculosis, antimalarials, Antibacterials	leaves, flowers	Bunyapraphatsara and Chokechareunporn, 1999; Farnsworth and Bunyapraphatsara, 1992 Yadav and Singh, 2010 Haw and Keng, 2003
	Antifungals, skin diseases Immunomodulatory Antiscorbutic Local anesthetics Digestive	leaves	Tiwari et al., 2011 Sahu et al., 2011 Leng et al., 2011; Sahu et al., 2011 Tiwari et al., 2011 Leng et al., 2011; Sahu et al., 2011
	Obesity control (lipase inhibitor)	flowers	Yuliana et al., 2011
	Snake bite	whole plant	Tiwari et al., 2011
Dental	Toothache	leaves, flower	Haw and Keng, 2003; Tiwari et al., 2011
	Toothpaste	leaves	Savadi et al., 2010
	Periodontal disease	flower heads, roots	Abascal and Yarnell, 2001; Sahu et al., 2011; Shimada and Gomi, 1995
	Recurrent aphthous stomatitis	leaves	Abascal and Yarnell, 2010
Beauty care cosmetics	Fast acting muscle relaxant Anti wrinkle	whole plant	Belfer, 2007 Demarne and Passaro, 2009; Schubnel, 2007

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Table 2: Thai medicinal plants for dental application

Plant species family	Common name	Thai name	Part used	Treatment and usage
[1]*Barleria lupulina* Linn. Acanthaceae	Barleria	Saled-pang-porn	whole plant	toothache, oral ulceration, oral diseases
[1]*Cocos nucifera* Linn. Arecaceae	Coconut	Ma-phrao	oil from coconut shell, root	toothache
[1]*Helianthus annuus* Linn. Asteraceae	Sunflower	Tan-tawan	flower head	toothache
[1]*Averrhoa carambola* Linn. Averrhoaceae	Star fruit	Ma-fuang	fruit	toothache, scurvy, oral ulceration
[1]*Spilanthes acmella* Murr. Compositae	Paracress	Phak-krad	leaf, flower, root, whole plant	fever, toothache potential local anesthetic
[1]*Citrullus lanatus* Mats & Nakai Cucurbitaceae	Watermelon	Tang-mo	fruit	toothache, oral ulceration
[1]*Ocimum canum* L., O. *basilicum* L. Labiatae	Holy basil, Sweet basil	Mang-lak	whole plant	whole plant
[2]*Ocimum sanctum* Linn. Labiatae	Holy basil	Kra-prao	leaf	Toothpaste and mouthwash ingredients
[3]*Mentha cordifolia* Opiz.ex Fresen Labiatae	Kitchen mint	Sa-ra-nhae	leaf	toothache
[4]*Cinnamomum bejolghota* (Buch.-Ham.) Lauraceae	Cinnamon	Oub-choei	Root, bark	dissolve sputum, toothpaste, mouthwash and chewing gum ingredient
[2]*Cinnamomum camphora* (Linn.) Presl Lauraceae	Camphor tree	Kara-boon	leaf, seed	toothache, gingivitis
[1]*Tinospora crispa* Linn. Menispermaceae	Tinospora stem	Bora-ped	leaf, flower	periodontitis, toothache
[1]*Streblus asper* Lour. Moraceae	Toothbrush tree	Khoi	stem bud	toothache, gingivitis, antimicrobial in oral cavity, toothpaste ingredient
[1]*Psidium guajava* Linn. Myrtaceae	Guava	Fah-rhang	leaf, fruit, leaf	toothache, halitosis, scurvy, gingivitis, toothpaste ingredient
[2]*Syzygium aromaticum* Linn.*Eugenia caryophyllus* (Sprenge) Bullock et Harrison Myrtaceae	Clove	Kan–plu	flower	toothache, scurvy, toothpaste and mouthwash ingredients
[2]*Murraya paniculata* Linn. Jack Rutaceae	Orange jasmine	Keaw	leaf	toothache
[1]*Solanum melongena* Linn. Solanaceae	Egg plant	Ma-khau-yao	stem, root, flower	toothache, oral ulceration

[1](Thiengburanathum, 1999), [2](Boonkird et al., 1982),[3] (Matchacheep, 1991), [4] (Thanaphum and Muengwongyard, 2006)

In addition, phytosterols (e.g. *β*-sitosterol, stigmasterol, *α*- and *β*-amyrins), essential oils (e.g. limonene and *β*-caryophyllene), sesquiterpenes, *α*- and *β*-bisabolenes and cadinenes, flavonoid glucoside and a mixture of long chain hydrocarbons (C22-C35) were reported (Sahu et al., 2011; Tiwari et al., 2011).

In recent years, other bioactive metabolites **10 – 15** (Figure 2) have been isolated from the aerial part of *S. acmella*, namely vanillic acid (**10**), *trans*-ferulic acid (**11**), *trans*-isoferulic acid (**12**), scopolelin (**13**), 3-acetylaleuritolic acid (**14**) and *β*-sitostenone (**15**) (Prachayasittikul et al., 2009b).

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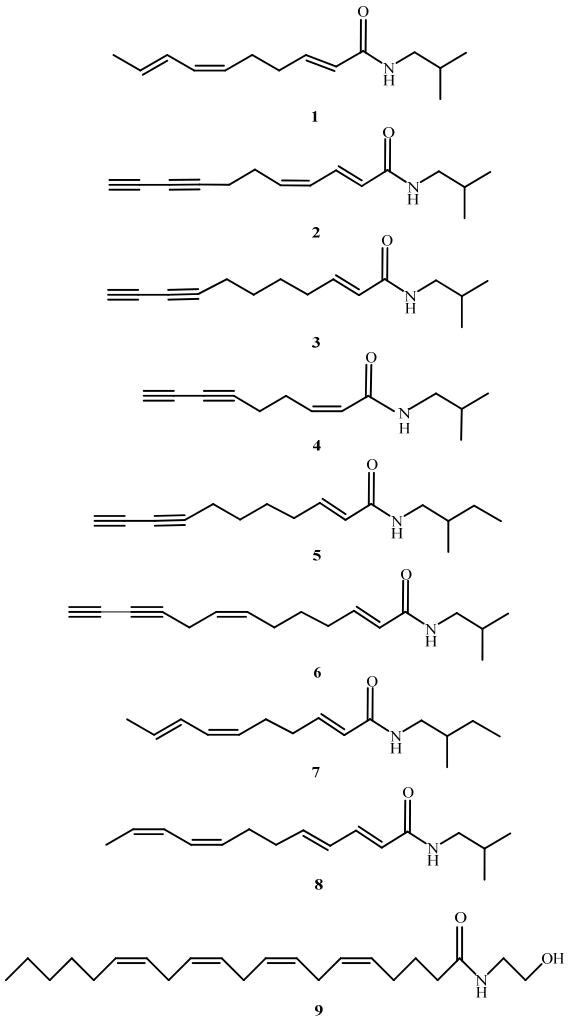

Figure 1: Structure of spilanthol and derivatives

Table 3: Spilanthol from plant species

Tribe	Genus	Species
Ecliptinae Less	*Welelia*	*parviceps*
Galinsoginae B. *and* H.	*Acmella*	*ciliata* *oleracea* *oppositifolia* *radicans*
Zinniinae B. *and* H.	*Heliopsis*	*longipes*

Bioactivity

The *Spilanthes* genera have been used for the treatment of various disorders including life-threatening diseases. Diverse pharmacological activities of this plant species were previously reported (Sahu et al., 2011; Tiwari et al., 2011). Selected bioactivities of *S. acmella* are summarized below.

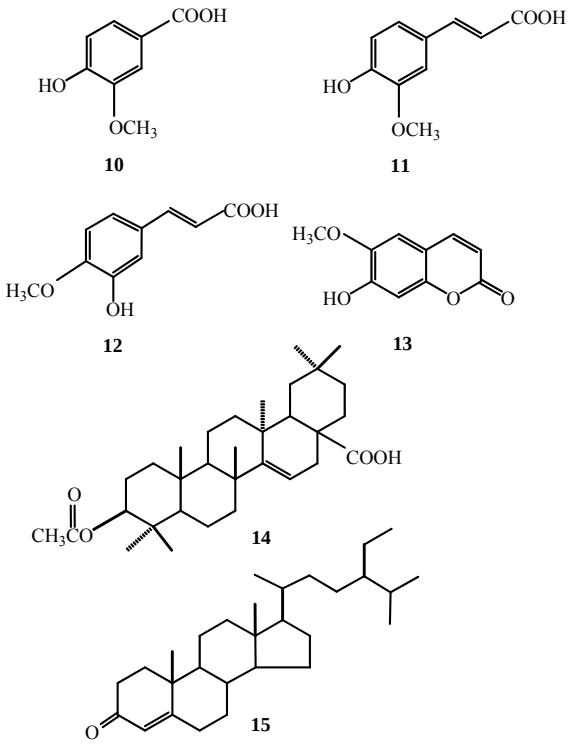

Figure 2: Bioactive metabolites isolated from *S. acmella*

Antipyretic activity

Many medicinal plants have long been used as antipyretics, e.g. *S. acmella* (flower and aerial aqueous) extracts (Chakraborty et al., 2010). In general, pyrexia or fever is caused by a secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy and other diseases (Elumalai et al., 2012). These impacts initiate the formation of pro-inflammatory mediators or in particular cytokines (i.e. interleukin 1β, α, β, and TNF-α). This results in an increase of prostaglandin E2 (PGE2) synthesis and ultimately increases the body temperature (Elumalai et al., 2012). The studies showed that *S. acmella* (aerial aqueous extract) displayed antipyretic activity against Brewer's yeast-induced pyrexia. The antipyretic activity of the plant species can be attributed to flavonoids (Narayana et al., 2001; Trease and Evans, 1972), which were predominant inhibitors of either cyclooxygenase (COX) or lipoxygenase (LOX) (Sadavongvivad and Supavilai, 1977). Flavonoids are known to target prostaglandins in the late

phase of acute inflammation and pain (Chakraborty et al., 2004).

Antiinflammatory activity

Spilanthol is the main constituent isolated from many parts of *S. acmella* such as flower 85 % EtOH extract(Wu et al., 2008), root hexane extract (Wagner, 1989) and also from other plants such as *Heliopsis longipes* root EtOH extract (Hernández et al., 2009). Traditional usages of *S. acmella* flowers have been reported as antiinflammatory agent (Sharma, 2003). Previous investigations demonstrated that spilanthol exerted antiinflammatory action *via* inhibition of NF-κB pathway; afforded reduction in mRNA level and protein expression of COX-2 and iNOS; and also induced free radical scavenging activity (Wu et al., 2008). Most antiinflammatory medicinal plants possessed LOX and COX inhibitors such as Asteraceae, Apiaceae, Lamiaceae and Fabaceae (Schneider and Bucar, 2005). Plant species affording such properties is the *H. longipes* root extract and its isolated spilanthol (Hernández et al., 2009). The antiinflammatory activity of spilanthol can be attributed to its dual inhibition of COX and LOX owing to the similar structures of spilanthol and arachidonic acid, in which the arachidonic acid is a precursor of prostaglandin and leukotriene syntheses (Hernández et al., 2009). Interestingly, the *H. longipes* extract displayed stronger antiinflammatory activity than that of the spilanthol. This was possibly due to synergistic effects of the containing compounds in the plant extract (Hernández et al., 2009). Moreover, EtOH extract from the leaves of *S. acmella* exhibited significant antiinflammatory activity against acute (carragenan induced rat paw edema method), sub-acute (granuloma pouch method) and chronic (adjuvant arthritis method) inflammation (Barman et al., 2009) but has been shown to be less than that of aspirin. The observed antiinflammatory activity originates from the inherent flavonoids that are found in the plant extracts (Chakraborty et al., 2004). Mechanisms of acute inflammation consists of two phases involve with histamine, serotonin and kinin, released in the first hour (Ganesh et al., 2008) and with prostaglandin-like substances that are released in the second and third hours. Therefore the antiinflammatory action of *S. acmella* may take part in the later phase *via* inhibition of COX enzyme (Brooks and Day, 1991).

A recent study in 2012 has shown that triterpenoids, namely β-sitosterol and β-sitostenone isolated from *Leucosidea sericea* (Rosaceae), exhibited antiinflammatory activity *via* inhibitions of COX-1 and COX-2. The study employed indomethacin as a standard drug and the results showed that β-sitosterol displayed stronger antiinflammatory activity than the standard drug (Nair et al., 2012).

Analgesic activity

A number of antitoothache plants has been recognized, *S. acmella* is one of these plants that has been used in pain relief. The studies showed that *S. acmella* EtOH leaves extracts exerted significant centrally (e.g. tail flick method) and peripherally (e.g. Writhing test) analgesic activities (D' Armour and Smith, 1941; Witkin et al., 1961). The mechanism of action was possibly due to the presence of flavonoids in the plant extract (Chakraborty et al., 2004) which decreases prostaglandins, PGE2 and PGF2 that are known to be involved in pain perception (Jyothi et al., 2008). In addition, cold aqueous extract of *S. acmella* flowers also displayed antinociceptive activity against persistent pain and antihyperalgesic activity. The mechanism of action was possibly through inhibition of prostaglandins by spilanthol-containing extract (Ratnasooriya and Pieris, 2005).

Another study of antitoothache plant *H. longipes* revealed that it was used as local anesthetic and analgesic in Mexican indigenous medicine and the results showed that its stem acetone extract and spilanthol from root displayed dose-dependent antinociceptive effect in mice as assessed by Writhing and capsaicin tests (Déciga-

Campos et al., 2010). Possible mechanisms of action of the spilanthol and *H. longipes* extract may be attributed to the activation of opioidergic, serotoninergic and GABAergic systems as well as the K^+channel opening facilitated by nitric oxide (NO) induced cGMP production (Acosta-Madrid et al., 2009; Rios et al., 2007). Partial participation of cGMP and K^+channel in the antinociceptive activity of spilanthol was proposed for several drugs (Bermúdez-Ocaña et al., 2006; Hernandez-Pacheco et al., 2008; Ortiz et al., 2006).

Local anesthetic activity

S. acmella is known to be constituted of pungent alkamide-like spilanthol that causes numbness and tingle. Local anesthetic activity was studied in animal models through intracutaneous wheal in guinea pigs and plexus anesthesia in frogs (Chakraborty et al., 2010). *S. acmella* aerial aqueous extract exhibited significant activity that could be due to the presence of alkamides (Chakraborty et al., 2010). However, its onset of action was slower than that of xylocaine, the standard drug.

The well-recognized local anesthetics are comprised of mostly amide compounds such as xylocaine (lidocaine). Its mechanism of action involves the blockage of voltage-gated Na^+ channels. By the same analogy, the alkamides of *S. acmella* extracts produced local anesthetic action presumably through the blockage of Na^+ channels. Isobutylamide and piperovatine of other antitoothache plant (*Piper piscatorum*) were reported to display local anesthetic activity through the same mechanism of action (McFerren et al., 2002).

Antimicrobial activity

Ethyl acetate (EtOAc) and methanol (MeOH) extracts from the leaves of *S. acmella* exhibited the strongest antimicrobial activity among the tested extracts using the well diffusion method against *Klebsiella pneumonia*e (Arora et al., 2011). The EtOAc extract had two-fold higher activity than that of doxycycline, the standard drug, whereas the MeOH extract showed comparable activity with doxycycline. This could be due to the fact that the plants contain flavonoids, tannins, and other phytochemicals, which are well-known antimicrobials. On the other hand, aerial parts of EtOAc and MeOH extracts from *S. acmella* tested by the agar dilution method were shown to be inactive antimicrobials whereas its chloroform ($CHCl_3$) extract displayed antimicrobial activity against *Streptococcus pyogenes* with MIC of 256 µg/mL (Prachayasittikul et al., 2009b). In addition, hexane and $CHCl_3$ extracts exhibited antifungal activity (MIC 256 µg/mL) against *Saccharomyces cerevisiae* (Prachayasittikul et al., 2009b).Moreover, isolated fractions of $CHCl_3$ and EtOAc extracts selectively inhibited the growth of *Corynebacterium diphtheria*e NCTC 10356 with MIC range 64-256 µg/mL (Prachayasittikul et al., 2009b). Another study showed that leaves/flowers MeOH extract of the plant species displayed no antimicrobial action (disk diffusion method) (Nanasombat and Teckchuen, 2009).

Medicinal plants for treatments of oral cavity infections, dental caries and periodontal diseases were reported (Rosas-Piñón et al., 2012). The most frequently used were species from many families: Myrthaceae (17.8 %), Punica (15.1 %), Compositae (11.3 %), Asteraceae (9.7 %), Piperaceae (8.6 %), Anacardiaceae (7.3 %), Fagaceae (6.9 %), Labiateae (5.4 %), Leguminosae (5.1 %), Butalaceae (0.5 %) and others (6.4 %). Dental caries and periodontal diseases are two major dental pathologies affecting humankind that arises from colonization and accumulation of oral microorganisms especially *Streptococcus mutans* and *Porphyromonas gingivalis* (Rosas-Piñón et al., 2012). Antibacterial activity of Compositae plant extracts against oral microorganisms is shown in Table 4 (Rosas-Piñón et al., 2012). The data showed that *S. mutans* was the most sensitive to plant extracts while *P. gingivalis* was the most resistant (Rosas-Piñón et al., 2012).

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Antifungal activity

Several parts of *S. acmella* were tested for antifungal activity (Table 5) and the studies showed that *S. acmella* leaves (EtOAc and aqueous) extracts exhibited better antifungal activity than the standard drug (fluconazole) against *Rhizopus arrhigus* and *Rhizopus stolonifer* (Arora et al., 2011).The leaves extract also displayed weak activity against *Aspergillus niger* and *Penicillium chrysogenum* (Arora et al., 2011). The whole plant $CHCl_3$ extract was shown to be active antifungal against opportunistic fungal infection (e.g. *Microsporum gypseum* and *Cryptococcus neoformans*) in AIDS patients (Phongpaichit et al., 2005). *S. acmella* flower head petroleum ether extract exerted antifungal activity against *A. niger*, *A. parasiticus*, *Fusarium moniliformis* and *F. oxysporium* (Rani and Murty, 2006). The antifungal activity of *S. acmella* extracts may be due to the presence of spilanthol and alkamides (Nakatani and Nagashiwa, 1992), non-volatile sesquiterpenoids and saponins (Krishnaswami et al., 1975; Mukharya and Ansari, 1986). In addition, aerial parts of *S. acmella* extracts (hexane and $CHCl_3$) exhibited activity against *Saccharomycese cerevisiae* (Prachayasittikul et al., 2009b).

Table 4: Antibacterial activity[a] of Compositae plants

	S. mutans		*P. gingivalis*	
Compositae	H_2O extract	EtOH extract	H_2O extract	EtOH extract
Cirsium mexicanum DC.	>1000	>1000	>1000	>1000
Iostephane heterophylla (Cav.) Benth.	67.5	125	125	250
Heterotheca inuloides Cass.	125	32.5	500	125
Coreopsis mutica DC.	250	62.5	>1000	>1000
Calendula officinalis	125	250	500	500

[a]Antibacterial activity was determined by MIC value (μg/mL), using microdilution method. H_2O denoted as an aqueous

Antimalarial activity

S. acmella is a traditional medicine used in Africa and India for the treatment of malaria (Spelman et al. 2011). Pharmacological study showed that spilanthol (**1**) and acetylenic alkamide (undeca-2*E*-ene-8,10-diynoic acid isobutylamide or UDA) (**3**), isolated from the root EtOH extract of *S. acmella*, displayed antimalarial activity against two strains of *Plasmodium falciparum* (PFB strain originated from Brazil and chloroquine resistant, K1 strain originated from Thailand). Both compounds had a reported antimalarial activity with IC_{50} in the range of 5.8-41.4 μg/mL in which the spilanthol was the most potent compound. It was reported that semi-purified compounds of *S. acmella*, isolated by centrifugal partition chromatography (CPC) and electrospray ionization-ion trap-time of flight-mass spectrometry (ESI-IT-TOF-MS), showed significantly higher antiplasmodial activity as indicated by the lower IC_{50} value (Mbeunkui et al. 2011). This could be a result from synergistic effects of *N*-alkylamides in the tested compounds. Moreover, regenerated *S .acmella* (*in vitro*) root hexane extract exhibited 100 % larvicidal activity affording the lowest values of LC_{50} and LC_{90} against malaria and filarial vectors (Pandey and Agrawal, 2009). It was suggested that the regenerated plant species contained higher active principle content than those that are field grown. In addition, the studies demonstrated the potential of *S. acmella* for the treatment and prevention of malaria (Bae et al., 2010).

Antioxidant activity

Antioxidant activity of *S. acmella* extracts obtained from polar and nonpolar solvents were investigated. It was found that *S. acmella* flower EtOAc extract displayed the highest free radical scavenging activity (DPPH and ABTS assays) when compared to the other tested extracts (Wu et al., 2008). On the other hand, leaves and flowers of *S. acmella* MeOH extracts showed weak antioxidant activity

(Nanasombat and Teckchuen, 2009). The aerial parts of *S. acmella* were also investigated (Prachayasittikul et al., 2009b; Wongsawatkul et al., 2008). The tested extracts (hexane, $CHCl_3$, EtOAc and MeOH) exhibited antioxidant activity as indicated by DPPH and SOD assays. The EtOAc and MeOH extracts were shown to be the most potent antioxidants (DPPH). This could be due to the presence of phenolic and coumarin compounds that are present in the extracts (Prachayasittikul et al., 2009b). In addition, fractions isolated from $CHCl_3$ extract exerted potent SOD activity, which may be attributed to the presence of triterpenoids, stigmasterol and its glucosides (Prachayasittikul et al., 2009b). Interestingly, the fractions from the MeOH extract which displayed strong and potent antioxidant activity as well as being shown to exhibit antimicrobial activity (Prachayasittikul et al., 2009b). Other medicinal plants with antioxidant activity also showed antimicrobial actions, e.g. *Saraca thaipingensis* (Leguminosae) (Prachayasittikul et al., 2012), *Polyalthia cerasoides* (Annonaceae) (Prachayasittikul et al., 2010a) and *Hydnophytum formicarum* Jack. (Rubiaceae) (Prachayasittikul et al., 2008).

Vasorelaxant activity

S. acmella extracts were studied for their vascular effects using rat thoracic aorta (Wongsawatkul et al., 2008). The results showed that the tested extracts exhibited vasorelaxant activity *via* partial endothelium-induced NO and PGI_2 in dose dependent manner. EtOAc extract displayed immediate vasorelaxant and the most potent antioxidant (DPPH) activities. Similar vasorelaxant and antioxidant (SOD) activities were also observed in the $CHCl_3$ extract of the plant species (Prachayasittikul et al., 2009b; Wongsawatkul et al., 2008). These bioactivities can be attributed to the presence of phenolic and triterpenoids (Prachayasittikul et al., 2009b). The other plant species of Compositae, the *Eclipta prostrata* Linn. were also shown to possess vasorelaxant and antioxidant activities (Prachayasittikul et al., 2010b). In addition, analogs of nicotinic acid (vitamin B3) and orotic acid (vitamin B13) were reported to afford vasorelaxants and antioxidants (Prachayasittikul et al., 2010c, d).

The plant species have been used as a powerful aphrodisiac in traditional medicinal practice for cases of sexual deficiency or depressed desire as it has been shown to improve sexual function in man (Sharma et al., 2011). The study showed that *S. acmella* EtOH flower extract improved sexual

Table 5: Antifungal activity of *S. acmella*

Plant extract	Tested part	Method	*Microorganism*	Inhibition zone or MIC	References
EtOAc	leaves	well diffusion	*R. arrhigus*	23[a]	Arora et al., 2011
Aqueous	leaves	well diffusion	*R. stolonifer*	25[a]	
EtOAc	leaves	well diffusion	*A. niger*	16 [a]	
EtOAc, MeOH, petroleum ether	leaves	well diffusion	*P. chrysogenum*	14,12,15[a]	
Petroleum ether	flower heads	agar cup bioassay	*A. niger* *A. parasiticus* *F. oxysporium* *F.monilifermis*	20 [a] 18 [a] 23 [a] 21 [a]	Rani and Murty, 2006
$CHCl_3$	whole plants	modified agar dilution method	*M. gypseum* *C. neoformans*	256 [b] 128 [b]	Phongpaichit et al., 2005
Hexane,$CHCl_3$ EtOAc,MeOH	aerial parts	agar dilution method	*S. cerevisiae* inactive	256 [b] -	Prachayasittikul et al., 2009b

[a] inhibition zone (mm.), [b] MIC(μg/mL)

behavior. It was suggested that alkamides may mimic the action of testosterone or stimulate the secretion of testosterone. In addition, the contribution of NO in vaso-relaxation (Wongsawatkul et al., 2008) may be involved in enhancing sexual performance as penile erection is directly controlled by NO (Sharma et al., 2011). The study suggested possible development of *S. acmella* EtOH extract as therapeutics for stimulating male sexual activity (Sharma et al., 2011).

Moreover, *S. acmella* extract is an active component in body and beauty care cosmetics as a fast-acting muscle relaxant that may be essential in accelerating the repair of functional wrinkles as well as stimulate, reorganize and strengthen the collagen network and has thus been utilized for anti aging purposes in the form of anti wrinkle cream formulations (Prachayasittikul et al., 2009b). Other plant species such as the *Zanthoxylum bungeanum* fruit husks extract are known to be rich in spilanthol that has been found to exert anti wrinkle effect owing to its capacity to relax subcutaneous muscles and act as a topical-lifting agent for wrinkles (Artaria et al., 2011).

Diuretic activity

Naturally occurring diuretics such as caffeine are known to be present in coffee, tea and cola. So far in Ayurvedic practice, many indigenous drugs have been claimed to have diuretic effect. The study of *S. acmella* EtOH leaves extract revealed diuretic effect possibly arising from tannin, steroid and carotenoid (Vanamala et al., 2012). In addition, flower cold aqueous extract of the plant species exhibited strong diuretic activity (Kumar et al., 2010). The effect may be attributed to its alkaloids. It was suggested that the extract acted as a loop diuretic, which is the most powerful of all diuretics (Ratnasooriya et al., 2004). However, several other diuretic plants from different families have been reported to contain triterpenoids, steroids, saponins, alkaloids, flavonoids, phenolics, glycosides and bis-benzylisoquinolines (Vanamala et al., 2012).

Immunostimulant activity

S. acmella leaves have been used traditionally as tonic, treatment of rheumatism, gout and sialogogue as well as being claimed to possess immunostimulant activity (Savadi et al., 2010). The investigation was performed using various experimental models. The EtOH leaves extract showed significant immunomodulatory activity by increasing macrophage count with the maximum number of cells on the 15th day (Savadi et al., 2010). The leaves of *S. acmella* contained various compounds such as alkamides, pungent amides, carbohydrates, tannins, steroids, carotenoids, essential oils, sesquiterpenes and amino acids (Amal and Sudhendu 1998; Lemos et al., 1991; Nagashima and Nakatani, 1992; Nagashima and Nobuji, 1991; Tiwari and Kakkar, 1990). It was reported that spilanthol was involved in immune stimulation and attenuation of inflammatory response in murine Raw 264.7 macrophages (Wu et al., 2008). In addition, some alkamides are being consumed as to enhance immune response, for example, to relieve colds, respiratory infections and influenza (Rios, 2012).

Structure-activity relationship

As stated previously, *S. acmella* extract and its isolates (e.g. spilanthol, flavonoids and triterpenoids) are known to be involved in many bioactivities. Particularly, antipyretic, antiinflammatory and analgesic activities arise from the capacity of compounds to inhibit COX enzymes that lead to the inhibition of prostaglandin syntheses. The well-known drug such as aspirin has been used as a nonsteroidal anti-inflammatory drug, analgesics and antipyretics. Its mechanism of action had been proposed (Brenner and Stevens, 2010) to irreversibly inhibit COX enzymes that are homodimeric proteins containing two identical active sites with serine residues at positions 530 (COX-1) and 516 (COX-2),

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forming covalent bond. Ultimately, acetyl groups of the aspirin were added to COX enzymes *via* nucleophilic attack of serine (OH group) to electrophilic center (carbonyl group) of the drug (Figure 3).

Considering the structures of spilanthol, contains amide carbonyl group, flavonoid, coumarin and triterpenoid (*β*-sitostenone), which all have electrophilic centers that could interact with serine residues of COX enzymes. Thus, the possible mode of action of spilanthol is presumably a result from the addition of serine (OH) to the carbonyl group of spilanthol with subsequent loss of amine moiety as shown in Figure 4.

Based on the functional moiety of the compounds, therefore, similar enzymatic nucleophilic addition of serine (OH) to electrophilic carbonyl groups of coumarin and *β*-sitostenone could possibly be proposed. Besides the carbonyl group, an alcohol function of the compound such as *β*-sitosterol has been shown to be a stronger antiinflammatory agent than the *β*-sitostenone, and even stronger than the standard drug, indomethacin (Nair et al., 2012). This could be due to the loss of the OH group from *β*-sitosterol as H_2O molecules, thus forming carbocation (electrophilic center) that further reacted with the nucleophilic serine (OH) as described previously.

Another important correlation that has been observed for the compounds is aside from the antioxidant activity they also possess vasorelaxant activity (Prachayasittikul et al., 2010b; Wongsawatkul et al., 2008). This could be attributed to the fact that antioxidant compounds inhibited the formation of peroxynitrite as a result from the reaction of NO (as superoxide scavenger) with $O_2^{\cdot-}$ thus improving NO-induced vasorelaxation.

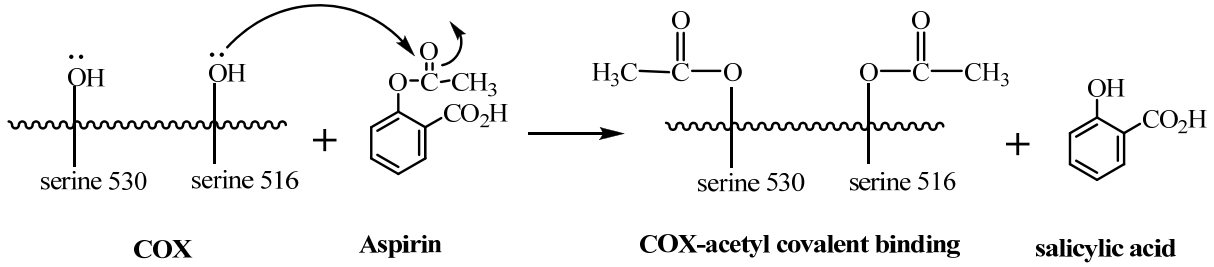

Figure 3: Proposed mechanism of action of aspirin

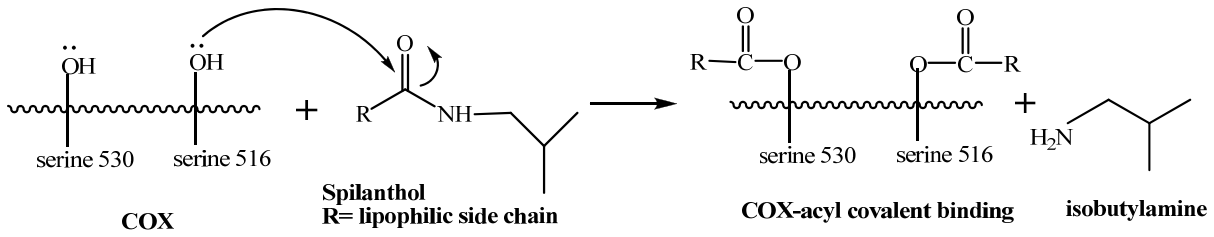

Figure 4: Possible mode of action of spilanthol

In addition, it has been observed that *S. acmella* extracts provided both antioxidant activity and antimicrobial action. So far, many other medicinal plants also showed such correlation (Prachayasittikul et al., 2008, 2012). It was reported that compounds with antimicrobial activity also displayed antioxidant activity as well (Suksrichavalit et al., 2008, 2009). Such activity relationship could be possibly explained by the fact that these compounds may enhance bacterial killing by synergistically converting superoxide radical to hydrogen peroxide (H_2O_2) in which accumulation of H_2O_2 exhibited harmful effect to bacterial cells as well as participating in the ultimate formation of hydroxyl radical through Fenton's reaction (Suksrichavalit et al., 2008).

Molecular modeling of vasorelaxant and antioxidant activities had been previously reported (Prachayasittikul et al., 2010c). It was found that dipole moment (μ) is a useful molecular descriptor for assessing the vasorelaxant and antioxidant activities where compounds with high μ correspondingly had high antioxidant (SOD) activity. This suggested that electron withdrawing group is crucial for superoxide scavenging (SOD) activity. The explanation is that molecules having high μ induce a positive charge that is highly capable to scavenge the superoxide anion (Prachayasittikul et al., 2010c; Worachartcheewan et al., 2012). Thus, the compound with high antioxidant activity facilitates the induced NO which plays the important role in vasorelaxation. The radical scavenging (DPPH) activity can be assessed from the ionization potential (IP) where low IP value is an indicator of good antioxidant activity as it has a higher probability of losing an electron in scavenging the radical (Prachayasittikul et al., 2010c).

Preparation and purification

A combination of bioactive compounds has been found in *S. acmella.* Especially, spilanthol is the most abundant alkamide accounting for the diverse bioactivities and its wide range of medicinal applications as well as its increasing demand for the market (Tiwari et al., 2011). Spilanthol is constituent in many parts of the plant species: flower heads, leaves, aerial parts, stems and roots. In general, chemical constituents can be isolated by conventional chromatography and identified by spectroscopic methods, NMR, IR, HPLC and LC-MS (Prachayasittikul et al., 2008, 2009a, 2010a; Tiwari et al., 2011).

To obtain a large-scale spilanthol with higher yields and purities, therefore more effective methods are required. Recently, supercritical fluid extraction (SFE) has been proven to be the most effective extraction process for spilanthol from all parts of *S. acmella* (e.g. flowers, leaves and stems) (Dias et al., 2012). The advantage of SFE provides ready-to-use product with contamination-free green extract and solvent independence (Dias et al., 2012). Previously, other plant species such as *S. americana* (Stashenko et al., 1996) and *Echinacea angustifolia* (Sun et al., 2002) were validated using the SFE method. It was found that the method was efficient for selective extraction of spilanthol from *S. americana* flowers and leaves.

High pressure liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS) was employed as a rapid and effective identification and quantification method for spilanthol from *S. acmella* (e.g. whole plants, leaves, flowers, stems and roots) EtOH extracts (Bae et al., 2010). Furthermore, the obtained spilanthol was shown to be stable in EtOH extracts for well over six months when even stored at room temperature (Bae et al., 2010).

CPC is another technique used for quantitative isolation of *N*-alkylamides from *S. acmella* MeOH flower extract (Mbeunkui et al., 2011). Structures of the isolates were identified by ESI-IT-TOF-MS and validated by ^{1}H-and^{13}C-NMR analysis. The CPC offered high recovery of the target compounds and high-throughput as compared with other traditional separation methods, for example, column chromatography and thin layer

chromatography. The study demonstrated the potential of CPC for large-scale isolation of major *N*-alkylamides from *S. acmella* (Mbeunkui et al., 2011).

***In vitro* micropropagation**

To date, *S. acmella*, with high medicinal values, is increasingly demanded worldwide as a plant-derived medicine (Tiwari et al., 2011). It has been recognized as one of the most important medicinal plants of the world (Singh and Chaturvedi, 2012a). However, *S. acmella* has been itemized as an endangered plant species due to the low rate of germination and poor vegetative propagation (Rios-Chavez et al., 2003), including limited availability of information of the biosynthetic pathway of alkamides (Tiwari et al., 2011).

To increase the supply of *S. acmella*, *in vitro* micropropagation has been recently proposed to be a reliable and routine approach for large-scale production (Sahu et al., 2011; Tiwari et al., 2011). The method is a useful tool for rapid cultivation of *S. acmella* which provides high yield and consistent production or quality of bioactive metabolites irrespective of seasons and regions (Singh and Chaturvedi, 2012a) as well as conservation of genetic fidelity, long term storage and cost effectiveness (Sahu et al., 2011). So far, a number of studies has been reported for successful *in vitro* micropropagation of *S. acmella* through leaf, axillary bud, and shoot tip (Sahu et al., 2011). The content of spilanthol was found to be higher than the mother plant or those that are field grown (Singh and Chaturvedi, 2012b). Importantly, the produced spilanthol (*in vitro*) showed strong (100 %) antilarvicidal activity against malaria and filarial vectors (Pandey and Agrawal, 2009). The methods employed different culture media, mostly using Murashige and Skoog media (MS) in combination with other growth regulators or auxins as shown in Table 6.

CONCLUSION

It is very fascinating that *S. acmella*, starting from the simple antitoothache plant to highly valuable annual herb, possesses multifunctional roles as indigenous medicine for therapeutics in health care, beauty care and cosmetics as well as health food or supplements enriched with numerous antioxidants. The most abundant isolates of the plant species were lipid alkamides, especially, the spilanthol along with other bioactive metabolites e.g. phenolic, flavonoid, coumarin and triterpenoid compounds.

Pharmacological studies revealed that such compounds exhibited an array of diverse bioactivities. Considering the data, some conclusions could be drawn that the *S. acmella* extracts and its constituting compounds such as spilanthol and flavonoids have been shown to possess inhibitory activity toward PG synthesis. It could be presumably proposed that these compounds share a common functional group with electrophilic center, interacting with COX enzymes through nucleophilic addition of serine residues. As a result, the syntheses of PG were inhibited subsequently contributing to the observed antiinflammatory, antipyretic and analgesic activities. In addition, spilanthol has been shown to reduce NO release and thereby inhibit inflammatory mediators and attenuating the expression of COX-2 and iNOS. This could be attributed to the immunostimulant activity of *S. acmella* in its traditional usages.

S. acmella exerted vasorelaxant and antioxidant activities, which is beneficial for its lifting effect as fast acting muscle relaxant in anti wrinkle and anti aging applications. The participation of NO in vasorelaxation makes *S. acmella* a powerful aphrodisiac in traditional medicine for improving sexual performance in men.

Table 6: *In vitro* production of *Spilanthes*

Plant species	Induction	Culture media	Method	References
S. acmella	[a]callus formations (cell biomass)	BAP;2,4D,NAA/MS	leaf disc explant (cell suspension culture)	Singh and Chaturvedi, 2012a
S. acmella	[a,b]shoots *via* direct organogenesis	BAP/MS BAP,NAA/MS BAP,IAA/MS	leaf disc explant	Singh and Chaturvedi, 2012b
S. acmella	[c] -	2,4-D/MS	callus cell suspension	Leng et al., 2011
S. acmella	plant flowers	BA,NAA/MS	leaf explant	Pandey et al., 2011
S. acmella	shoots flowerings	BAP/MS BAP,IAA/MS	cultured nodal re-generated shoot	Yadav and Singh, 2011
S. acmella	[d]shoot buds	BA,NAA/MS	seedling leaf ex-plant	Pandey and Agrawal, 2009
S. acmella	[e]shoot generations shoot tips	BAP/MS Sodium algena-te/$CaCl_2$	nodal segment algenate-encapsulated	Singh et al., 2009
S. acmella	shoots	BA, indole acetic acid/MS	leaf explant	Saritha and Naidu, 2008
S. acmella	multiple shoots	MS	nodal explant	Leng et al., 2004
S. acmella	multiple shoots	BA/MS	aseptic bud	Haw and Keng, 2003
S. calva	shoots	thidiazuron/MS	nodal segment	Tiwari et al., 2011
S. mauri-tiana DC	shoots	BA,NAA	axillary bud	Bais et al., 2002

[a]*In vitro* plant produced higher spilanthol than the mother plant (field grown). [b]Ploidy stability is similar to the field grown plant. [c]Spilnathol (*in vitro*) had similar retention time to the mother plant and flower head. [d]*In vitro* plant possessed strong larvicidal activity. [e]Generated shoots can be stored at 4 °C for 60 days.
BA = N^6- benzyladenine, BAP = N^6- benzylaminopurine, IAA = indole 3- acetic acid, MS = Murashige and Skoog medium, NAA = α-naphthalene acetic acid, 2,4-D = 2,4-dichlorophenoxy acetic acid

Structure-activity relationship of vaso-relaxant and antioxidant activities of nico-tinic acid derivatives were elucidated by molecular modeling. The studies provided insights on the essential molecular de-scriptors governing the observed biological activities. Such findings provide useful in-sights for the design and synthesis of ro-bust bioactive compounds.

To supply the market demand of *S. ac-mella* as a plant-derived medicine, its preparation, purification and *in vitro* prop-agation have been discussed herein.

In brief, it could be demonstrated that *S. acmella* is a medicinal plant enriched with compounds having high therapeutic value that can be further developed for applica-tions in medicines, health care, cosmetics, supplements and health food.

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