




## Review

**Phytochemical compounds and biological activity in Asparagus roots: a review**Hongxia Zhang,<sup>1\*</sup>  John Birch,<sup>1</sup> Jinjin Pei,<sup>2</sup> Zheng Fei Ma<sup>3</sup>  & Alaa El-Din Bekhit<sup>1\*</sup> <sup>1</sup> Department of Food Science, University of Otago, PO Box 56, Dunedin New Zealand<sup>2</sup> Shanxi Key Laboratory of Bioresources, Shanxi University of Technology, Hanzhong 723001, China<sup>3</sup> Department of Public Health, Xi'an Jiaotong-Liverpool University, Suzhou 215213, China

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**Summary** Asparagus roots (AR) contain many bioactive compounds and their use in Chinese and Indian traditional medicines is well documented. Both *in vitro* and *in vivo* studies have indicated AR extracts act as antidiabetic, antioxidant and hypolipidaemic and depict anti-inflammatory, antibacterial, antiviral, anticarcinogenic activities and also improve cardiovascular and oral health. The physiological effects against wide range of pathologies are due to the wide range of phytochemicals found in AR including polyphenols, saponins and polysaccharides. The aim of this review is to present an overview of the functional, medical and physiological properties of AR bioactives. This review considers the roots of several asparagus species and provides up-to-date information on *Asparagus officinalis* that has not been extensively reviewed before.

**Keywords** Asparagus roots, functional, medical and physiological properties.

**Introduction**

There are hundreds of asparagus species (Lee *et al.*, 1997) with the most common being *Asparagus officinalis* L. (green asparagus) that has nutritional and commercial importance as an edible plant. The plant is native to the Mediterranean and Western Asia and has been cultivated for more than 2000 years (McKinlay, 1992). Generally, green asparagus has a productive life of 10–15 years, after which it needs to be replaced with new asparagus seedlings to improve productivity. The edible shoots account for about 23.5% of the whole asparagus plant and the remaining by-products account for the majority (76%) of the asparagus plant, including roots that account for 15% of the plant. The *Asparagus officinalis* L roots traditionally have little commercial value and most are usually considered waste. Previous reports have shown that *A. officinalis* L roots contain significant antioxidant capacities (Symes *et al.*, 2018; Zhang *et al.*, 2018a), suggesting that *A. officinalis* L roots may serve as an excellent dietary source of natural antioxidants for health promotion (Fan *et al.*, 2015) and anti-inflammatory effects (Jang *et al.*, 2004). In ancient China, *A. officinalis* L was used as a traditional medicine for treatment of inflammation, cancer, infection

and coughs (Fan *et al.*, 2015). The nutritional, phytochemical and pharmacological properties of *A. officinalis* L roots have gained interest and have been investigated for their therapeutic properties and health promotion (Huang *et al.*, 2008) in order to utilise *A. officinalis* L roots and explore their potential to provide an extra income to farmers.

*Asparagus racemosus*, another asparagus species, is widely cultivated in India where its roots have been used for folk medicines. This species has received a lot of interest as a result of its therapeutic qualities (Bopana & Saxena, 2007), including antidepressant and antihepatotoxic activities (Singh *et al.*, 2009a,b; Dey *et al.*, 2013). Since ancient times, *A. racemosus* roots have been used in “Ayurveda, Unani and Siddha” medicine, with numerous beneficial effects in several diseases (Bopana & Saxena, 2007). Another asparagus variety, *Asparagus africanus* roots, has been widely used in folk medicine for its antiparasitic activity (Dikasso *et al.*, 2006), antiepileptic (Jalalpure *et al.*, 2009), antilithiatic (Goel *et al.*, 2006), antihepatotoxic (Muruganadan *et al.*, 2000), antidepressant (Singh *et al.*, 2009a) and antineoplastic (Rao, 1981) activities and to treat and cure stomach ulcers (Sairam *et al.*, 2003). It also exhibited neuroprotective activity (Parihar & Hemnani, 2004).

*Asparagus officinalis* L extracts could potentially be used as bioactive supplements because several

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important bioactive compounds have been reported in their extracts, including steroidal saponins (Huang *et al.*, 2008), rutin, inosine, quercetin and caffeic acid (Huang *et al.*, 2006; Symes *et al.*, 2018; Zhang *et al.*, 2018a), fumaric acid, isoferulic acid, ferulic acid, citric acid and asparagusic acid (Hartung *et al.*, 1990; Huang *et al.*, 2006). The major phenolic constituent detected in *A. officinalis* roots is controversial, with rutin or caffeic acid (Hartung *et al.*, 1990; Huang *et al.*, 2006) having been reported as the major phenolic present. Since AR extracts are rich in many bioactive compounds including steroidal glycosides, polycyclic alkaloids, terpenes and phenolics, they are used in traditional medicine (Fan *et al.*, 2015) and have been proposed as a source for pharmaceutical natural products (Tiwari, 2008) to meet consumer interest in natural products to maintain health and well-being (Viuda-Martos *et al.*, 2010).

The objective of this review is to present an overview of the functional, medicinal and physiological properties of AR extracts to provide a comprehensive account of potential uses of asparagus in food and health applications that could consequently have positive nutritional, economic and environmental outcomes. This review will critically evaluate the bioactive compounds and nutritional value of AR. Further, the impact of the roots' background (e.g. cultivar, environment and origin) will be examined to provide the best options towards industrial applications. The physiological and pharmacological activities of AR extracts, their mechanisms of action and their efficacy in various model systems will be evaluated.

## Phytochemistry of AR

### Chemical composition of AR

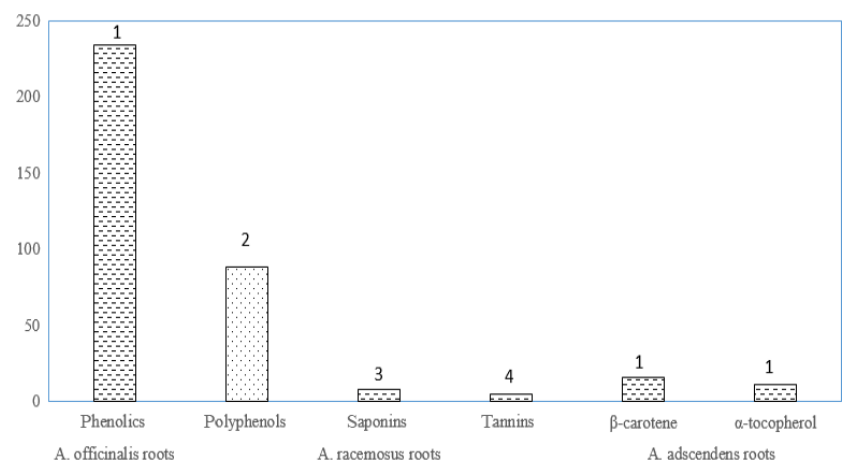
Different nutrients and bioactives can be found in different AR, depending on the cultivar and region

(Zhang *et al.*, 2018a). *Asparagus racemosus* roots contain 36.8%–52.89% carbohydrates, 2.95%–6.1% protein, 17.93% crude fibre, 4.1%–8.83% saponins, 5%–6.2% oil and 4.18% inorganic matter in addition to smaller amounts of vitamin C, flavonoids and polyphenols as shown in Fig. 1 (Mathew *et al.*, 2005; Velavan *et al.*, 2007). On the other hand, higher bioactive contents (18.20% total saponins, 14.86% total polysaccharides and 12.63% free amino acids) in *A. officinalis* extract after extraction with 75% ethanol and ultrasonic-assisted extraction was obtained and demonstrated a remarkable immune-enhancing function in Concanavalin A-induced proliferation of spleen lymphocytes (Tian *et al.*, 2013). Concanavalin A is lectin that acts as a mitogen for the stimulation of cells to present an immune response.

Kamat *et al.* (2000) investigated the polysaccharide composition of *A. racemosus* roots (MW, 2000 kDa) and found high amounts of galactose and glucose and smaller amounts of arabinose and other sugars (galactose 54%, glucose 28%, rhamnose 4%, xylose 5% and arabinose 8%). The contents of  $\beta$ -carotene, xanthophyll,  $\alpha$ -tocopherol and total phenolics in *A. adscendens* Roxb roots were 15.5 mg per 100 g, 0.10 mg per 100 g, 11.32 mg per 100 g and 234.5 mg per 100 g respectively (Prasad *et al.*, 2014). Collectively, these results demonstrate that AR are very rich in bioactives and the composition and contents of the bioactives vary among the different asparagus species and from AR in different studies (Mathew *et al.*, 2005; Velavan *et al.*, 2007; Zhang *et al.*, 2018a).

### Chemical composition of vitamin, microelement and free amino acid contents in AR (nutritional and antinutritional aspects)

Different levels of vitamins A, C, B<sub>1</sub>, B<sub>2</sub> and E, and folic acid can be found in various AR. Other phytochemical constituents in asparagus are arginine,



**Figure 1** Composition of Asparagus species root. [Colour figure can be viewed at wileyonlinelibrary.com]

tyrosine, asparagine, flavonoids (kaempferol, quercetin and rutin), essential oils, resin and tannins (Negi *et al.*, 2010). For example, *A. adscendens* Roxb roots are rich in vitamin C (Prasad *et al.*, 2014), an antioxidant compound that protects the human body against free radicals, strengthens the immune system and keeps human gums healthy (Balasubramani *et al.*, 2011). Dry *A. adscendens* Roxb roots provide 310.63–311.81 mg of vitamin C per 100 g (Prasad *et al.*, 2014). The recommended median daily amount of vitamin C is 500 mg for people (Shibata *et al.*, 1992); thus 160 g of AR can provide the recommended daily dose. However, since several other bioactives exist, extensive studies are required to fully understand their possible contribution to human health before recommending its regular consumption (Shibata *et al.*, 1992). Little is known about how many free amino acids are in AR and there are also discrepancies among the different cultivars. Che *et al.* (2013) reported that *A. officinalis* roots contained at least 15 free amino acids in *A. officinalis* roots by RP-HPLC, which was lower than that in *A. officinalis* top. These included aspartic acid, glutamic acid, asparagine, histidine, glutamine, arginine, serine, proline, glycine, alanine, methionine, cysteine, tryptophan, leucine, isoleucine, phenylalanine, tyrosine and lysine. It is suggested that different colours, and parts had significant effects on free amino acid content in *A. officinalis*.

### Antimicrobial properties

The use of natural antimicrobials from microbial, animal or plant origin can improve the shelf life of foods because of their bacteriostatic/fungistatic or bactericidal/fungicidal activity. Antimicrobials can be used to control microbial changes in food products such as off-odours and changes in colour and texture (Viuda-Martos *et al.*, 2010). The antibacterial activity of AR extracts has been widely reported, as depicted in Fig. 2. Several *in vitro* assays have shown bactericidal properties against several highly pathogenic and antibiotic-resistant organisms (Mandal *et al.*, 2000; Ravishankar *et al.*, 2012; Madikizela *et al.*, 2013). Seven compounds that have been isolated from *Asparagus acutifolius* roots exhibited significant antifungal activity against human pathogenic yeasts such as *C. albicans*, *C. glabrata* and *C. tropicalis* with minimum inhibition concentration (MICs) between 12.5 and 100  $\mu\text{g mL}^{-1}$  (Sautour *et al.*, 2007). The amphipathicity of these compounds explains their interactions with the cell membrane and thus present antifungal activity. According to Aggarwal *et al.* (2013), different extraction methods used to extract antibacterial substances revealed different antibacterial activities. Aggarwal *et al.* (2013) evaluated three different extraction methods: cold maceration method,

soxhlet extraction and reflux extraction for the extraction of bioactive compounds from *A. racemosus* roots and evaluated their antibacterial activities against five bacterial strains (*Escherichia coli* DH5a, *Pseudomonas aeruginosa* MTCC 1688, *Klebsiella pneumoniae*, *Enterococcus cloacae* and Gram-positive bacteria, *Staphylococcus aureus*). A methanolic extract of *A. racemosus* roots obtained by reflux extraction was found to possess significant antibacterial activity against *E. coli* DH5a and *P. aeruginosa* (MTCC 1688) with MICs of 0.25 and 0.5  $\text{mg mL}^{-1}$ , respectively, which is higher than that extracted from *A. racemosus* roots by a cold maceration method. The authors suggested that the reflux extraction temperature resulted in a higher extract yield than the cold maceration method (Aggarwal *et al.*, 2013). The principle component predicted to be involved in the antibacterial effects of *A. racemosus* roots extract is a cyclic hydrocarbon-9, 10-dihydropheanthrene (Aggarwal *et al.*, 2013). Uddin *et al.* (2012) also reported antibacterial activity of *A. racemosus* roots methanolic extract against eight bacterial strains, including *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas alkaligenes*, *Proteus* specie, *Shigella* species, *Salmonella typhi*, *Vibrio cholera* and *S. aureus*. Further, an ethanol extract obtained from *A. racemosus* roots that was tested for antimicrobial activity resulted in a spectrum of inhibition against *S. aureus*, *Bacillus subtilis*, *Staphylococcus wernerii*, *Pseudomonas putida*, *P. aeruginosa* and *Proteus mirabilis* (Ravishankar *et al.*, 2012). To elucidate the effects of extraction solvents on the antimicrobial activity of AR, Madikizela *et al.* (2013) investigated different extraction solvent extracts of *A. falcatus* roots for their inhibitory effects against *K. pneumoniae*, *Mycobacterium aurum* A+ and *S. aureus*. The 80% ethanol extract was more effective than the other extraction solvents (petroleum ether [PE], dichloromethane [DCM] and water) against *M. aurum* A+ and *S. aureus*. The values of MIC were 12.50, 3.13 and 12.50  $\text{mg mL}^{-1}$  respectively with PE extract, 12.50, 1.56 and 12.50  $\text{mg mL}^{-1}$  respectively with DCM extract, 6.25, 0.39 and 3.13  $\text{mg mL}^{-1}$  respectively with 80% ethanol extraction and 3.13, 2.56 and 1.56  $\text{mg mL}^{-1}$  respectively with water extraction (Madikizela *et al.*, 2013). The hydrophilic component of the functional groups available on the compounds available in the extracts interacts with the polar component of the microorganism membrane. The mechanism responsible for the lethal effects of phenolic compounds on microorganisms has been related to reactions with sulfhydryl groups of proteins and the unavailability of substrates to microorganisms, or to interference with bacterial protein secretions (Viuda-Martos *et al.*, 2010). Potduang *et al.* (2008) identified the presence of steroids-terpenes, alkaloids and flavonoids in an ethanol extract of *A. racemosus* roots by TLC and HPLC finger printing. In general, steroids-

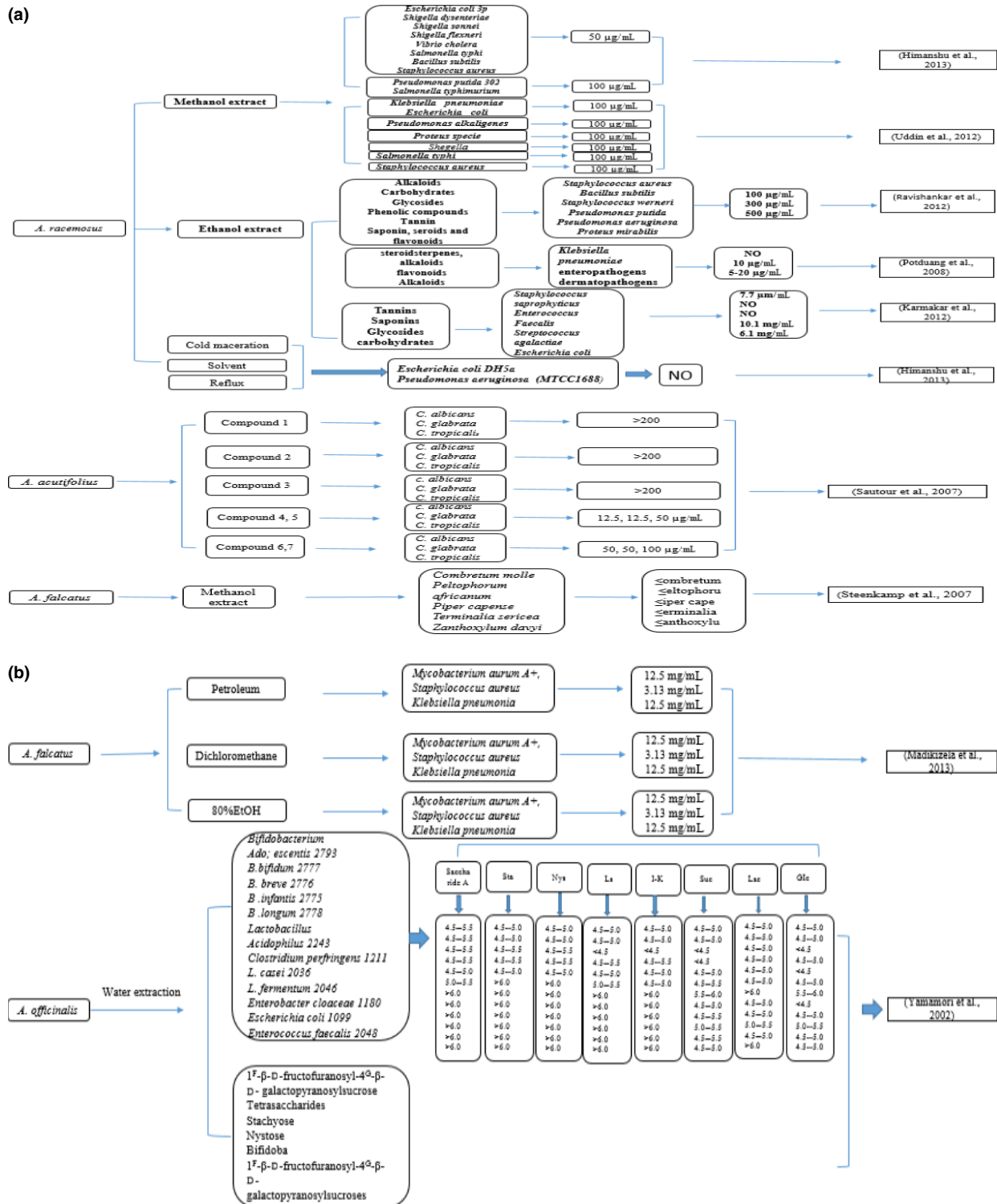


Figure 2 (a) Antimicrobial properties of Asparagus species roots and (b) Antimicrobial properties of Asparagus species roots. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 *In vitro* antioxidant activity of the root of *Asparagus* species

<i>In vitro</i> antioxidant activity									
Sample	DPPH	FRAP	ABTS	O <sup>2-</sup>	H <sub>2</sub> O <sub>2</sub>	Others	Extraction method	Extraction solvents	References
<i>A. officinalis</i> L	0.050 mmol TEAC per g	-	0.102 mmol TEAC per g	-	-	-	70 °C for 2 h in one-factor-at-a-time approach	50% acetone	Fan et al. (2015)
	0.056 mmol TEAC per g	-	0.127 mmol TEAC per g	-	-	-	-	50% ethanol	
	0.043 mmol TEAC per g	-	0.105 mmol TEAC per g	-	-	-	-	70% methanol	
	79% 50% 60%	17.15 mmol mg <sup>-1</sup> 4.7 mmol mg <sup>-1</sup> 7.9 mmol mg <sup>-1</sup>	-	82% 50% 18%	-	-	80% ethanol for 2 h	n-butyl alcohol Water	Feng et al. (2011)
<i>A. officinalis</i> and <i>A. filicinus</i> mix	3.2 mg mL <sup>-1</sup> 1.2 mg mL <sup>-1</sup>	14.00 mg 6.94 mg	MC -	4.48 mg mL <sup>-1</sup> 12.33 mg mL <sup>-1</sup>	NO (6.832 mg mL <sup>-1</sup> ) -	SO (4.482 mg mL <sup>-1</sup> ) 12.331 mg mL <sup>-1</sup>	Soxhlet extractor	Ethanol Methanol	Subba & Mandal (2015)
<i>A. racemosus</i>	0.4 mg mL <sup>-1</sup>	1.73 mg	3.10 mg mL <sup>-1</sup>	0.37 mg mL <sup>-1</sup>	29.608 mg mL <sup>-1</sup>	0.369 mg mL <sup>-1</sup>	-	Water	
	0.6 mg mL <sup>-1</sup>	3.54 mg	-	0.83 mg mL <sup>-1</sup>	9.224 mg mL <sup>-1</sup>	0.833 mg mL <sup>-1</sup>	-	Butanol	
	0.26 mg mL <sup>-1</sup>	1.06 mg	4.278 mg mL <sup>-1</sup>	0.78 mg mL <sup>-1</sup>	5.046 mg mL <sup>-1</sup>	0.782 mg mL <sup>-1</sup>	-	Acetone	
	1.5 mg mL <sup>-1</sup>	9.33 mg	1.272 mg mL <sup>-1</sup>	3.38 mg mL <sup>-1</sup>	-	3.379 mg mL <sup>-1</sup>	-	Chloroform	
	0.47 mg mL <sup>-1</sup>	1.89 mg	-	1.20 mg mL <sup>-1</sup>	3.516 mg mL <sup>-1</sup>	1.197 mg mL <sup>-1</sup>	-	Ethyl acetate	
	2.156 mg mL <sup>-1</sup>	9.311	0.976 mg mL <sup>-1</sup>	0.171 mg mL <sup>-1</sup>	2.990 mg mL <sup>-1</sup>	-	-	Hexane	
	0.653 mg mL <sup>-1</sup>	2.994	0.328 mg mL <sup>-1</sup>	0.089 mg mL <sup>-1</sup>	-	-	-	Heptane	
	1.094 mg mL <sup>-1</sup>	4.955	-	0.091 mg mL <sup>-1</sup>	-	7.147 mg mL <sup>-1</sup>	-	Benzene	
	106.44 µg mL <sup>-1</sup>	-	-	-	-	-	-	Methanol	Hossain et al. (2012)
	78.15 µg mL <sup>-1</sup> 273.31 µg mL <sup>-1</sup>	- -	- -	- -	- -	- -	- -	Soxhlet extractor	Methanol Ethanol Petroleum ether
<i>A. racemosus</i>	712.61 µg mL <sup>-1</sup> 26.3%	80 mM Fe <sup>2+</sup> per g	-	-	-	-	4 h in shaking technique	Chloroform 60% methanol	Jain et al. (2011)
<i>A. racemosus</i>	30%	-	-	-	-	-	24 h by shaking	80% methanol	Ghimire et al. (2011)
<i>A. racemosus</i>	613.9 µg mL <sup>-1</sup> 348.6 µg mL <sup>-1</sup>	1655.9 µg mL <sup>-1</sup> -	- -	- -	- -	- -	24 h, conventional extraction	Methanol Absolute n-butanol	Shah et al. (2013)

Table 1 (Continued)

In vitro antioxidant activity										
Sample	DPPH	FRAP	ABTS	O <sup>2-</sup>	H <sub>2</sub> O <sub>2</sub>	Others	Extraction method	Extraction solvents	References	
	>1000	-	-	-	-	-	-	Aqueous extraction		
<i>A. racemosus</i>	86.84 µg mL <sup>-1</sup>	20-88.63 µg mL <sup>-1</sup>	-	-	20-46.51 µg mL <sup>-1</sup>	-	48 h with absolute methanol	Absolute methanol	Rathanavel & Arasu (2014)	
<i>A. racemosus</i>	46.25 µg mL <sup>-1</sup>	-	-	-	-	-	-	Methanol extraction	Dohare et al. (2011)	
<i>A. racemosus</i>	381.91 µg mL <sup>-1</sup> ;	-	-	-	IC50 for tyrosinase inhibition 7.98 mg mL <sup>-1</sup> .	LC50 of brine shrimp 2189.49 µg mL <sup>-1</sup>	-	-	Potduang et al. (2008)	
<i>A. adscendens</i> Roxb	0.202 mg mL <sup>-1</sup>	9.4 µmol Fe (II) per g	-	-	-	-	45 °C for 2 h	80% methanol	Guleria et al. (2013)	

terpenes, alkaloids and flavonoids are assumed to be toxic to microorganisms as they create stable complexes with cellular materials, mainly with proteins and to a lesser extent with carbohydrates or physiological metal ions (such as Fe and Cu). Potduang *et al.* (2008) examined the antimicrobial effects of ethanolic extract of *A. racemosus* roots against pathogenic microbes (16 species, 18 strains) and found the MICs were in the range of 5–20 mg mL<sup>-1</sup>. For enteropathogens, the MIC was between 10 and 20 mg mL<sup>-1</sup>, whereas the MIC was 10 mg mL<sup>-1</sup> for the pneumonia-causing bacterium *K. pneumoniae*. Similarly, Karmakar *et al.* (2012) reported that 80% ethanol extract of *A. racemosus* roots was active against the gram-positive strains of *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Streptococcus agalactiae*, *S. pyogenes* and the gram-negative bacterial strains *Shigella boydii*, *S. sonnei*, *S. dysenteriae*, *S. flexneri*, *P. aeruginosa* and *E. coli*. They found that ethanol extract at the concentration of 500 µg disc<sup>-1</sup> resulted in moderate antibacterial activity against *S. saprophyticus*, *E. faecalis*, *S. agalactiae* and *E. coli*, with zones of inhibition of 7.77 ± 0.37, 6.07 ± 0.06, 10.10 ± 0.11 and 6.00 ± 0.04 mm, respectively, while a concentration of 250 µg disc<sup>-1</sup> of the ethanol extract did not show any inhibition against the tested bacterial strains. Not all of the crude AR extracts were effective against bacterial activities. For example, a study by Steenkamp *et al.* (2007) extracted *A. falcatus* thumb roots with methanol and water for screening against bacteria such as *Combretum molle*, *Peltophorum africanum*, *Piper capense*, *Terminalia sericea* and *Zanthoxylum davyi* but there was no inhibition found against these bacteria.

#### Antioxidant effects

Herbal medicine considers that the efficiency and safety for protection against cancer and vascular diseases and for scavenging of free radicals depends on the antioxidants in natural products (Muhammad *et al.*, 2016). The antioxidant activity of AR extracts extracted with ethanol, methanol and acetone were evaluated using an ABTS (2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolourisation assay and higher activity was found in the hydrophilic fractions of AR (Shah *et al.*, 2013; Fan *et al.*, 2015; Subba & Mandal, 2015). The antioxidant activity of AR samples appeared to be related to the phenolic and peptide contents of the extracts. The antioxidant levels of lipophilic and bound extracts of AR are rarely evaluated. Generally, the hydrophilic fractions are widely extracted by polar solutions as ethanol, methanol and water, while the lipophilic extracts require the use of non-polar solutions such as n-hexane (Tables 1 and 2). Extraction of bound phytochemicals is facilitated by acidic, alkaline or enzymatic

Table 2 Investigation of the *in vivo* antioxidant activity from asparagus roots

In vivo antioxidant activity (experimental model)							
Sample (root)	Biological activity	Animal	Method	Extraction	Dosage	Duration	References
<i>A. pubescens</i> <i>Bak</i>	Contraceptive activity	Immature albino female mice, rats and rabbits	Studying reproductive hormones (estrogenic, anti-oestrogenic, progestational and antiprogestational) oestrous cycles.	Methanol	Dose-dependent (0.5–1.5 g kg <sup>-1</sup> ) body	4–14 gestational periods	Nwafor et al. (1998)
<i>A. africanus</i>	Antiparasitic activity	Male Swiss albino mice	Inhibited the growth of <i>P. berghei malaria parasite</i>	80% Methanol	600 mg kg <sup>-1</sup>		Dikasso et al. (2006)
<i>A. pubescens</i>	Anti-inflammatory and antinociceptive activity	Adult albino mice and rats	Weight and length acetic acid-induced writhing, formalin-induced pain licking and hot plate-induced pain.	Methanol	0.25–1.5 g kg <sup>-1</sup>	21–23 12	Nwafor & Okwuasaba (2003); Nwafor et al. (1998).
<i>A. cochinchines</i>		Specific pathogen-free 5-week-old male	Skin thickness and tissue weight, inflammatory cytokine production, neutrophil-mediated myeloperoxidase (MPO) activity and various histopathological indicators.	70% Ethanol	200 mg kg <sup>-1</sup>	7 days	Choo et al. (2009)

solutions. Higher ABTS and DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging effects were found in the lipophilic extracts than the hydrophilic extracts of AR (Gan et al., 2017).

Asparagus roots were extracted with different organic solvents to evaluate the antioxidant activity of extracts using ABTS. The study reported that acetone, ethyl acetate, chloroform, methanol, butanol, water and ethanol extractions of a mixture of *A. officinalis* and *A. filicinus* roots in a Soxhlet extractor were also evaluated by ABTS and the result indicated that acetone extracts (IC<sub>50</sub> of 0.037 mg mL<sup>-1</sup>) had higher antioxidant activity, while water extracts (0.249 mg mL<sup>-1</sup>) had the least (Subba & Mandal, 2015). Further, a study by Shah et al. (2013) reported ABTS radical scavenging activity of IC<sub>50</sub> > 1000 µg mL<sup>-1</sup> with absolute methanol, 871.9 µg mL<sup>-1</sup> with n-butanol and >1000 µg mL<sup>-1</sup> with water at room temperature for 24 h. Although the ABTS scavenging assay is widely employed for the evaluation of free radical scavenging activity, the confirmation of antioxidant effects still needs to be further validated by other antioxidant methods (such as superoxide anion scavenging activity and FRAP). Due to soluble phenolic compounds that are found in cell vacuoles, highly polar organic solvents are widely used to extract antioxidant compounds, particularly lignin and flavonoids (Fan et al., 2015). Subba & Mandal (2015) used superoxide anion radical scavenging activity (O<sup>2-</sup> radical scavenging) and reported IC<sub>50</sub> (mg mL<sup>-1</sup>) of superoxide scavenging assay (SO) with water, acetone, butanol, ethyl acetate, chloroform, ethanol and methanol in a mixture of *A. officinalis* and *A. filicinus* roots in a Soxhlet extractor was 0.37, 0.78, 0.83, 1.20, 3.38, 4.48 and 12.33 mg mL<sup>-1</sup>, respectively, suggesting that water was the best extraction solvent. Rathanavel & Arasu (2014) reported much lower IC<sub>50</sub> (20–46.51 µg mL<sup>-1</sup>) for *A. racemosus* root methanolic extract in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical scavenging assay. The antioxidant activity appears to be affected by the solvents, sample cultivars and region where roots were obtained from and is closely correlated to extraction conditions (Jain et al., 2011; Rathanavel & Arasu, 2014).

The effect of the solvent on the antioxidant activity of an AR mixture (*A. officinalis* and *A. filicinus*) was investigated by Subba & Mandal (2015) using FRAP (ferric reducing antioxidant power) assay. The FRAP value of 1.06 mg ascorbic acid equivalent (AAE) per mg, 1.73, 1.89, 3.54, 6.94, 9.33 and 14.00 mg AAE per mg was found with acetone, water, ethyl acetate, butanol, methanol, chloroform and ethanol respectively. AR was extracted with different concentrations of methanol and absolute methanol to study its antioxidant capacity by FRAP measurement. Additionally, different varieties of AR presented varied antioxidant activities under the same extraction conditions in their

study. In another study, (Jain *et al.*, 2011) reported a FRAP of 80  $\mu\text{M Fe}^{2+}$  per g from *A. racemosus* roots extract with 60% methanol in the traditional extraction method, while Guleria *et al.* (2013) reported a FRAP value of 8.7  $\mu\text{mol Fe (II)}$  per g) with 80% methanol in *A. racemosus* roots and a value of 9.4  $\mu\text{mol Fe (II)}$  per g of *A. adscendens* Roxb roots with 80% methanol at 45 °C for 2 h extraction in a conventional extraction method, which further confirmed the values of FRAP are affected by the type of materials. Comprehensive fractionation of Thai *A. racemosus* was carried out by Kongkanermit *et al.* (2011) resulted in antioxidant activity (determined as DPPH radical scavenging activity and reported as  $\text{EC}_{50}$ ) of aqueous and hydro-alcoholic fractions that had  $\text{EC}_{50}$  of 500–600  $\mu\text{g mL}^{-1}$  which was much lower than that found with ascorbic acid (1.5  $\mu\text{g mL}^{-1}$ ). A 70% methanol *A. racemosus* extract was fractionated by methanol, ethyl acetate, n-Butanol, and water-precipitated materials were examined for their antioxidant activities using DPPH radical scavenging activity (Acharya *et al.*, 2012). The ethyl acetate fraction had the highest antioxidant activity followed by the methanol fraction. The lowest fraction was found in the water-precipitated fraction.

Generally, strong polarity solvent yields give higher antioxidant activity than low polarity solvents (Hossain *et al.*, 2012). In liquid-solvent extraction processes, the principle “like dissolved like” is the main driver of compound extraction and thus targets compounds with polarity values similar to the polarity of solvent. Few studies have reported the  $\text{IC}_{50}$  values of residues of *A. officinalis* L and *A. racemosus* roots as well as a mixture of *A. officinalis* and *A. filicinus* roots (Hossain *et al.*, 2012; Fan *et al.*, 2015; Subba & Mandal, 2015), which can supply a good basis for the effect of the polarity of the solvent on antioxidant activity in *A. officinalis* roots. Further study by Rathanaivel & Arasu (2014) also reported that the  $\text{IC}_{50}$  of DPPH radical scavenging activity was 86.84  $\mu\text{g mL}^{-1}$  of *A. racemosus* root in soxhlet extracted successively for 48 h with absolute methanol at room temperature, which was lower than that of  $\text{IC}_{50}$  (46.25  $\mu\text{g mL}^{-1}$ ) in a Dohare *et al.* (2011) study. It is suggested that the different values of  $\text{IC}_{50}$  are contributed by the composition of flavonoids in AR from the different regions. While an  $\text{EC}_{50}$  of DPPH radical scavenging activity of 0.209  $\text{mg mL}^{-1}$  was shown in dry weight of *A. racemosus* roots and 0.202  $\text{mg mL}^{-1}$  in *A. adscendens* Roxb roots with 80% methanol extraction at 45 °C for 2 h by shaking extraction methods (Guleria *et al.*, 2013). Their results suggest that the extraction parameters and various species of AR used are more important in extracting antioxidant compounds from AR, which should be taken into consideration in further studies.

The antioxidant activity of *A. officinalis* L root (AR) cultivars from China and New Zealand was affected by the extraction solvent system (Zhang *et al.*, 2018b). Nine solvents (water, methanol, ethanol, isopropyl alcohol, acetone, n-butyl alcohol, ethyl acetate, chloroform and petroleum ether) were used to investigate the optimal extraction solvent that generated the maximum total phenolics (TPC), total flavonoids (TFC), total saponins (TSC) and caffeic acid (CA) contents as well as maximum antioxidant activities using ferric reducing antioxidant power (FRAP), 2, 20-azinobis-(3- diphenyl-ethylbenzothiazoline-6-sulphonic acid) radical cation (ABTS) and  $\beta$ -carotene bleaching activity assays. Chinese yellow AR had the highest TPC, TFC, TSC and CA contents and the most efficient solvents for the extraction of these bioactives was ethanol and methanol. The authors further optimised the extraction parameters using a Box-Behnken design (BBD) (Zhang *et al.*, 2018a). The best extraction conditions for ethanol as extraction solvent were (51 °C, extraction time of 73.02 min, 75.23% ethanol concentration and 1:50 solid: liquid ratio) that resulted in total flavonoids content (TFC) of 17.1  $\text{mg RE per g}$ , total phenolic content (TPC) of 35.2  $\text{mg GAE per g}$ . Maximum antioxidant activities (2,2-diphenyl-1-picrylhydrazyl scavenging activity = 78.1%; 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonate scavenging activity = 61.2%; ferric reducing antioxidant power assay = 1.69  $\mu\text{mol g}^{-1}$ ,  $\beta$ -carotene bleaching activity = 76.0% and superoxide anion radical scavenging capacity = 70.2%) were obtained at extraction temperature of 50 °C for 78.5 min, using 70% ethanol at solid: liquid ratio of 1:40. A comparable methanolic extraction resulted in optimum extraction conditions (51 °C for 75 min, 75% methanol concentration 1:50 at solid: liquid ratio) resulted TFC of 13.4  $\text{mg RE per g}$  and TPC of 26.2  $\text{mg GAE per g}$ . The maximum antioxidant activities (DPPH radical scavenging activity = 42.69.9%), ABTS radical scavenging activity = 52.0%, FRAP activity = 0.59  $\mu\text{mol g}^{-1}$ ), and  $\beta$ -carotene bleaching activity = 71.2% and superoxide anion radical scavenging capacity = 41.6% were obtained at extraction temperature of 50 °C for 76.5 min using 80% methanol at 1:50 solid: liquid ratio.

## Pharmacological activities

### Aphrodisiac and contraceptive activity

The term aphrodisiac originates from the Greek word *Aphrodite*, eulogising the Greek goddess of love and romance. In modern times, this term has been used for substances that enhance sexual activity and are helpful in treating sexual dysfunction (Thakur *et al.*, 2009). Aphrodisiac activity of aqueous extracts of *A. racemosus* roots administered to rats for 28 days studying sexual behaviour in male albino rats at a dose of



200 mg kg<sup>-1</sup>, compared with a control group (untreated rats) in Swiss albino male rats, found an increase in body and reproductive organ weights. The effects might be due to testosterone-like effects of the *A. racemosus* roots extract. In another study, antifertility activity was investigated in adult and young immature albino female mice, rats and rabbits by administering a methanol extract of *Asparagus pubescens* Bak roots for 4–14 gestational periods and studying reproductive hormones (oestrogenic, anti-oestrogenic, progestational and antiprogestational) oestrous cycles and number of litters produced. *Asparagus pubescens* Bak roots extracts delayed the oestrous cycle at dose-dependent (0.5–1.5 g kg<sup>-1</sup> body weight) manner and enhanced the period of the dioestrous phase. Numbers of litters were also reduced. A study of oestrous cycles showed that *A. pubescens* Bak roots extracts changed the secretion of hormones as gonadotropin and oestradiol. Another study by Tafesse *et al.* (2006) reported that *A. africanus* roots extract indicated antifertility activities of 60% with aqueous and 40% with ethanol, respectively, by gavages to rats at a dose of 300 mg kg<sup>-1</sup> of body weight. The extraction of *A. africanus* roots potentiated acetylcholine significantly ( $P < 0.05$ ) and induced uterine contractions in a concentration-dependent manner. It was concluded that *A. pubescens* Bak roots extracts have an antifertility effect due to delaying the oestrous cycle and disturbing hormone secretion (Nwafor *et al.*, 1998; Tafesse *et al.*, 2006).

#### Anti-ageing and enhancing memory

Lipofuscin, a waste product of human and animal ageing metabolisms accumulated in cells, can lead to cell death and tumour formation. A study by Liu *et al.* (1996) reported that aqueous asparagus extract increased the SOD (superoxide dismutase) activity and reduced the lipofuscin concentration in *Drosophila melanogaster*. A study by Ojha *et al.* (2010) investigated the use of *A. racemosus* roots methanol extract to reverse memory deficits (amnesia) in mice induced by scopolamine (0.4 mg kg<sup>-1</sup>, i.p.) and sodium nitrite (75 mg kg<sup>-1</sup>, i.p.), which increased EPM (elevated plus maze) and MWM (Morris water maze) in short- and long-term memory tests. Memory deficit mice were fed two doses of extracts (75 and 150 mg kg<sup>-1</sup>, i.p.) for seven consecutive days. After that, the mice recovered from the amnesia and significantly improved EPM and MWM performance at a dose of 150 mg kg<sup>-1</sup>, i.p. Therefore, it is suggested that AR has the potential to relieve memory deficits.

#### Antiepileptic and antidepressant activity

In folk medicine, AR is used for its antiepileptic activity (Jalalpure *et al.*, 2009). The authors prepared

methanol, chloroform and aqueous extracts of *A. racemosus* roots and tested these on seizures induced by maximal electroshock (MES) and pentylenetetrazole (90 mg kg<sup>-1</sup>.p.o) at a dose rate of 200 mg kg<sup>-1</sup>.p.o in rats. While the methanolic extract resulted in a significantly lower time taken for hind limb extension (7 s) compared to the aqueous extract (8 s), chloroform extract (9 s) and control (14 s). In addition, the methanol extracts also had a significantly longer time for delaying the onset of convulsions than the control (495 s vs. 147 s), suggesting that *A. racemosus* roots have anticonvulsant properties. The authors suggested that methanolic extract of AR had the most significant effects on antiepileptic activity because of the presence of steroids, saponins and triterpenoids in AR.

Asparagus roots are also used in treating psychological disorders such as depression. A study by Singh *et al.* (2009a) evaluated various concentrations of methanolic extracts of *A. racemosus* roots to relieve depression in rats. Rats were given different concentration of AR extracts (100, 200 and 400 mg kg<sup>-1</sup> body weight) for 7 days before undergoing learned helplessness testing (LH) and forced swim testing (FST). Rats fed methanolic AR extracts had a significantly lower immobility period during FST and escaped failures in LH compared to controls, suggesting that AR possesses antidepressant activity (Singh *et al.*, 2009a).

#### Antiparasitic activity

Malaria is a major world public health problem, causing an estimated 1 to 2 million deaths per year and an annual incidence of 300–500 million clinical cases (Dikasso *et al.*, 2006). More than 2 billion people are at risk of infection from it due to increasing drug resistance and it is becoming more difficult to treat malaria (Dikasso *et al.*, 2006). The root of *A. africanus* was extracted with 80% methanol and tested against *Plasmodium berghei*, a chloroquine-sensitive strain maintained at the Ethiopian Health and Nutrition Research Institute (EHNRI) Laboratory Animal Sanctuary, in male Swiss albino mice. It was concluded that methanol extract of *A. africanus* roots inhibited the growth of *P. berghei* significantly with 46.12% inhibition at a dose of 600 mg kg<sup>-1</sup> and could therefore be used to treat malaria (Dikasso *et al.*, 2006).

#### Anti-inflammatory and antinociceptive activity

Development of cheap, safe herbal medicine from herbaceous plants for anti-inflammatory treatment has become more and more of interest because of the side effects and high cost of non-steroidal medicine (Hussain *et al.*, 2015). To evaluate anti-inflammatory and antinociceptive activity, chemical, thermal-induced pain and fresh egg albumin-induced inflammation and

pentylentetrazol (PTZ)-induced convulsion in rodents was applied to study the effects of methanolic extracts of *A. pubescens* roots. *Asparagus pubescens* root has a long history as a traditional medicinal for the treatment of various gastrointestinal disorders and inflammatory pains (Nwafor *et al.*, 1998; Nwafor & Okwuasaba, 2003). Nwafor & Okwuasaba (2003) studied methanolic extract of *A. pubescens* roots in adult albino mice, induced pain chemically and thermally, at a dose-dependent level (0.25–1.5 g kg<sup>-1</sup> body weight) where it was suggested that *A. pubescens* roots significantly inhibited acetic acid-induced writhing, hot plate-induced pain and formalin-induced pain licking inflammation. The extracts significantly ( $P < 0.02$ – $0.001$ ) enhanced the latencies of clonic and tonic convulsions and also delayed mouse mortalities based on constituents including saponins, tannins, flavonoids and steroids (Nwafor & Okwuasaba, 2003).

Paw oedema was induced by carrageenan in Wistar albino rats and a mercury displacement technique was employed to study the anti-inflammatory effect of 3.59% of crude saponins extraction from *A. africanus* Lam by TLC. The intraperitoneal LD<sub>50</sub> was found to be 1264.9 mg kg<sup>-1</sup> and the percentage of inhibition of crude saponins extraction was significantly ( $P < 0.05$ ) reduced in the rat paw-oedema or oedema (55%), as compared to ketoprofen (Lek, Yugoslavia: 10 mg kg<sup>-1</sup>) (63%) after 4 h in an anti-inflammatory study. It is suggested that *A. africanus* Lam. has significant anti-inflammatory properties due to its saponin content (Hossain *et al.*, 2012).

### Conclusion and future prospects

Among the plants of the family Asparagaceae, the versatile species of asparagus roots have captured greatest attention due to their pharmaceutical properties and their intensive folk medicinal uses. AR is highly regarded by folk healers for the treatment of cancer, diabetes, snakebites, diarrhoea, asthma, inflammations, ulcers, fevers and wounds. The antidepressant, antinociceptive, anti-inflammatory, antioxidant, hepatoprotective, antimicrobial, hypolipidaemic and hypoglycaemic activities of AR are other useful activities that have been under consideration. Given the diverse uses and the interest in natural nutraceutical trends, there is a need for more understanding of AR compositions and the impact of extraction parameters and methods, including conventional extraction methods and modern extraction methods on the properties and composition of their extracts. Most studies have focused on the antioxidant activity, phytochemical composition and pharmacological activities of *A. racemosus* roots, while other cultivars such as *A. officinalis* L are often ignored. The commercial importance of this plant means that large amounts of roots will be

available for viable commercial use. Information on the bioactives in this by-product can potentially improve the economics of the production for farmers and provide opportunities for further utilisation.

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