A review on phytochemical and pharmacological properties of Holy basil (*Ocimum sanctum* L.)

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ABSTRACT

Ocimum sanctum Linn. commonly known as *Holy Basil* or *Tulsi* is an Ayurvedic herb of Southeast Asia with a long history of traditional use. The culinary, medicinal and industrial importance of this plant led to explore its chemical and pharmacological properties. Here, we provide a comprehensive review on scientific findings of *O. sanctum* chemical constituents and their related anticancer, antioxidant, anti-inflammatory, antistress, γ-irradiation protection, antidiabetic and antileishmanicidal activities. More than 60 chemical compounds have been reported from *O. sanctum*, including phenolics, flavonoids, phenyl propanoids, terpenoids, fatty acid derivatives, essential oil, fixed oil, and steroids. The pharmacological activities of *O. sanctum* compounds reflect their medicinal importance and in the standardization of medicinal products. This compilation will be helpful in the development of new active principle and nutraceuticals in the area of drug resistance and emerging chronic disease vectors.

1. Introduction

The genus *Ocimum* belongs to the family Lamiaceae, comprises about 68 species indigenous to tropical regions of Asia, Africa and, central and south America (ThePlantList, 2013). *Ocimum sanctum* Linn. (*Os*) synonym *Ocimum tenuiflorum* L. (Lamiaceae), the most prominent species of the genera is cultivated worldwide for its medicinal, perfumery, religious, ceremonial, food and essential oil importance (Nadkarni, 1976). *Os* is a short-lived perennial shrub of 30–60 cm height with hairy stems and sparsely hairy leaves, which distributed in the Himalayas up to an altitude of 6000 feet (Watt, 1972). This aromatic shrub is commonly known as *Holy Basil* or *Tulsi* and identified as two common cultivars, *Rama Tulsi* with green leaves and *Krishna Tulsi* with purple leaves (Vani et al., 2009; Darrah, 1974). *Os* have been reported for antidiabetic, wound healing, antioxidant, radiation protective, immunomodulatory, antifertility, anti-inflammatory, antimicrobial, antistress and anticancer activities (Gholap and Kar, 2004; Vats et al., 2004; Udupa et al., 2006; Trevisan et al., 2006; Gupta et al., 2006; Geetha and Vasudevan, 2004; Yanpallewar et al., 2004; Bhartiya et al., 2006; Subramanian et al., 2005; Mukherjee et al., 2005; Godhwani et al., 1988; Ahmed et al., 2002; Kelm et al., 2000; Karthikeyan et al., 1999; Prashar et al., 1998; Prakash and Gupta, 2000; Singh et al., 2005). The toxicity studies suggest that *Os* is a nontoxic herb and safe to human use (Gautum and Goel, 2014; Sadashiv, 2010). The essential oil is one of the chemosystematic features of *Os* and a

good natural source of eugenol. *Os* essential oil has commercial importance in various industries including pharmaceutical, cosmetics and food as an antiallergic and antimicrobial agent (Kumar et al., 2010).

The present review on *Os* aims to provide a comprehensive study on its traditional uses, chemical constituents, nutritional values and pharmacological activities of *Os* secondary metabolites. So far, no such systematic study has been carried out on the commercially and medicinally important herb *Os*. Hence, this study on phytochemical constituents and their reported pharmacological activities will serve as a chemical database for future research as well as enable to understand the research gap and outlook for future prospects.

2. Traditional uses and ayurvedic recommendations

Os, known as *Tulsi* (the incomparable one, Hindi) has been described as *Rasayana* drug in the ancient texts of Ayurveda including Charak Samhita, Susrut Samhita and Rigveda (3500–1600 BCE) to treat cough, respiratory disorders, poisoning, impotence and arthritis (Bano et al., 2017). It is considered as one of the sacred plants in India. *Os* is used as a nervine tonic, adaptogen, improving health during cancer and has beneficial effects in stress release (Chulet and Pradhan, 2009; Balachandran and Govindarajan, 2005). The therapeutic potential of *Os* has been well documented in Ayurveda and Siddha for healing properties as well as in Greek, Roman and Unani system of medicines for the treatment of skin diseases, common cold, headaches, coughs, malarial

Table 1
Traditional uses of *O. sanctum*.

Plant parts (preparation used)	Ethnomedicinal uses	Region/Country	References
Fresh leaf with water	Enhancing mental power	Himachal Pradesh (India)	Vidyarthi et al. (2013)
Leaves with *Bruguiera gymnorrhiza* and coconut oil (pounded and rubbed on body)	Renovating from tiredness	Nicobar Island (India)	Dagar (1989)
Leaves pounded with onion bulbs (juice taken orally)	Cough, cold and headache	Tamil Nadu (India)	Muthu et al. (2006)
Leaves	Cough, cold, leg swelling and fever	Bangladesh	Chowdhury and Koike (2010)
Leaves (juice)	Cough, cold, bronchitis and gastric disorders	Bangladesh	Sharkar et al. (2013)
Whole plant	Cough, cold, headache, nausea, fever and skin diseases	Chuadanga, (Bangladesh)	Rahman et al. (2013)
Leaves pounded with garlic, leaves of *Achyranthes aspera* and pepper	Typhoid fever	Andhra Pradesh, India	Reddy et al. (1988)
Leaves pounded with fruits of *Tricosanthes diocia*, flowers of *Leucas indica* and leaves of *Aristolochia bracteata*	Typhoid fever	Andhra Pradesh (India)	Reddy et al. (1989)
Leaf decoction with flower heads of *Leucas cephalotes*	Fever	Makawanpur (Nepal)	Bhattarai (1991)
Leaf decoction with *Piper nigrum* and palmgur	Fever	India	Nazar et al. (2008)
Leaves paste with black pepper	Diarrhea and fever	Central Himalaya (India)	Kandari et al. (2012)
Leaves (juice)	Diarrhoea and dysentery	Tripura (India)	Sen et al. (2011)
Dried leaves with ghee	Dysentery, colic and piles	Central Himalaya (India)	Kandari et al. (2012)
Leaves (paste and decoction)	Stomach disorder, inflammations and wound cuts	Arunachal Pradesh (India)	Namsa et al. (2011)
Leaves (crushed and filtered extract)	Stomach ache and head ache	Assam (India)	Sajem and Gosai (2006)
Flowers juice with honey, ginger and onion juice	Bronchitis	India	Watt (1972)
Leaves (juice)	Bronchitis and catarrh	India	Watt (1972); Sharkar et al. (2013)
Dried leaves (vegetable)	Blood purification	Central Himalaya (India)	Kandari et al. (2012)
Leaves (juice)	To treat ringworm	Uttar Pradesh (India)	Siddiqui et al. (1989)
Leaves crushed in goat's urine and mixed with coconut oil	Skin allergy	Karnataka (India)	Shivanna and Rajakumar (2011)
Plant (paste)	Skin infection	Tripura (India)	Sen et al. (2011)
Leaves pounded with *Catharanthus roseus* leaves and mild heated	Ear boils	Karnataka (India)	Shivanna and Rajakumar (2011)
Leaves powder with honey	Diabetes	Assam (India)	Chakravarty and Kalita (2012)
Leave, flower top and roots (juice)	Antidote in snake poisoning	India	Watt (1972)
Leaves paste	Antidote for scorpion bite	Andhra Pradesh (India)	Reddy et al. (1988)

fever, diarrhoea, constipation and as an antidote for snake bite (Mondal et al., 2009; Uma Devi, 2001; Javanmardi et al., 2002). *Os* leaves have expectorant, carminative, refrigerant, febrifuge, laxative properties and their infusion is used as a stomachic in gastric disorders of children (Watt, 1972). Juice of fresh *Os* leaves is used as the first-aid remedy for earache. *Os* seeds are mucilaginous and demulcent and useful in the treatment of genitor-urinary disorders (Watt, 1972).

Os can be consumed as herbal tea, decoction (leaves and roots) to treat cough, cold and malarial fever (Prakash and Gupta, 2005). The paste of green or dried powdered leaves is used to treat ring-worm, skin diseases and vitalizing effect, whereas essential oil as larvicidal (Nadkarni, 1976). In Ayurvedic Pharmacopeia of India (1999), *Os* is recommended to treat pratishyaya (common cold), hikka (respiratory disorders), kasa (cough), aruci (loss of taste), kustha (skin disorders), krimiroga (treatment of worms) and parsva sula (chest pain) with a therapeutic dose of 2–3 g leaf powder. The available literature on the traditional uses of *Os* is limited to Asian countries, which compiled in Table 1.

3. Nutraceutical value (minerals, pigments and mucilage)

Minerals in routine intake of diet play an important role in food and nutraceutical industry. Herb *Os* has been used to add distinctive flavor in food and as a home remedy in various health conditions. The recent growing interest on the nutraceutical values of *Os* revealed that it is a rich source of vitamins, minerals, fat, protein, polysaccharide, fiber, pigments and mucilage (Pattanayak et al., 2010; Koche et al., 2011; Vidhani et al., 2016; Gowrishankar et al., 2010; Pachkore and Dhale, 2012). The macro and micro contents of *Os* are compiled in Table 2. The elemental analysis on macro and micro contents of *Os* leaves using Laser Induced Breakdown Spectroscopy (LIBS) and Inductively Coupled Argon Plasma Atomic Spectroscopy (ICAP-AES) techniques revealed the presence of almost all nutritionally important elements and interestingly

high concentration of potassium (10521.477 ± 391.7 mg/kg leaves) (Tripathi et al., 2015). Presence of high concentration of potassium and lighter elements like C, H, O and N suggest the application of *Os* in maintaining electrolytic balance and source of organic compounds, respectively.

Os contains vitamin A, vitamin C, β-carotene, chlorophyll, insoluble oxalates, protein (30 Kcal), fat (0.5 g), carbohydrate (2.3 g), minerals and other phytonutrients. Each 100 g of leaf contain vitamin C (83 µg), carotene (2.5 µg), Ca (3.15%), P (0.34%), Cr (2.9 µg), Cu (0.4 µg), Zn (0.15 µg), V (0.54 µg), Fe (2.32 µg) and Ni (0.73 µg) (Pattanayak et al., 2010). Bhattacharya et al. (2014) analyzed the antioxidant contents in *Os* leaves and found the total carotenoid content (19.77 ± 0.01 g/100 g), total phenolic content (2.09 ± 0.10 g/100 g) and total flavonoid content (1.87 ± 0.02 g/100 g) of dry weights. The presence of ascorbic acid (8.21 mg/100 g), riboflavin (0.06 mg/100 g) and thiamine (0.3 mg/100 g) contents further suggest that *Os* leaves can intake as a dietary supplement, an alternative economic source of vitamins and natural antioxidant.

Basil seed gum or mucilage is composed of two major components (i) an acid stable core gluco mannan and (ii) α-linked xylan including acidic side chains at C-2 and C-3 of xylosyl residues in acid-soluble portion (Naji-Tabasi and Razavi, 2017). The seed mucilage of *Os* (yield ∼30%), is a natural polymer that contains hexouronic acid (27.25%), pentoses (38.9%) and ash (0.2%) (Khare, 2016). *Os* seed mucilage has shown protein and amino acids on phytochemical evaluation, and possess swelling index 20 ml (water) with low ash value (Kadam et al., 2012). These physicochemical properties of mucilage direct towards its pharmaceutical excipient potential.

4. Chemical constituents of *Os*

Os leaves are rich in volatile oil (0.7%), phenolics, flavonoids, neolignans, terpenoids and fatty acid derivatives. *Os* seeds contain fixed

Table 2
Nutritional contents of *O. sanctum*.

Plant part	Nutritional composition (% of dry weight)						Mineral contents (ppm)			References
	Protein	Lipid	Carbohydrate	Fiber	Moisture	Ash	Ca	P	Zn	
Leaves	–	–	–	–	–	10.13	–	–	0.15	Narendhirakannan et al. (2005)
	12.30	3.00	77.70	7.00	83.55	18.35	–	–	–	Koche et al. (2011)
	4.93 ± 0.03	3.12 ± 0.28	27.23 ± 1.92	16.81 ± 1.25	31.35 ± 1.04	14.21 ± 1.50	18900	–	36	Kashif and Ullah (2013)
	0.80 ± 0.001	0.90 ± 0.45	2.10 ± 2.80	9.80 ± 0.80	5.30 ± 0.3	2.50 ± 0.08	450	–	713	Shafqatullah et al. (2013)
	20.84	–	–	10.0	11.44	–	1.8%*	1.0%*	0.49	Barua et al. (2015)
	20.64 ± 1.47	3.60 ± 0.08	39.58 ± 2.09	11.45	–	–	41204.1	–	32.38 ± 1.42	Vidhani et al. (2016)
	2.38%	3.20	66.35	–	6.20	10.42%	–	–	800	Wisdom et al. (2016)
Stem	9.25	2.75	68.05	18.30	88.30	20.15	–	–	–	Koche et al. (2011)
	1.10 ± 0.001	1.10 ± 0.60	2.20 ± 2.30	12.20 ± 1.40	6.60 ± 0.30	2.60 ± 0.10	450	–	747	Shafqatullah et al. (2013)
Seed	–	–	–	–	–	–	–	–	–	Pachkore and Dhale (2012)
Whole plant	–	–	–	–	–	–	4031 ± 102	–	–	Gowrishankar et al. (2010)
	–	–	–	–	–	–	35.9–19502	–	1.0–0.8	Pachkore and Dhale (2012)

Mineral contents (ppm)		Water soluble vitamins (ppm)				Calorefic value (kcal/100 g)	References
Fe	Cu	Vit B1	Vit B12	Vit C	Niacin		
2.32	0.4	–	–	–	–	–	Narendhirakannan et al. (2005)
–	–	–	–	–	–	–	Koche et al. (2011)
546	47850	–	–	24.1	–	–	Kashif and Ullah (2013)
354	31	–	–	310	–	–	Shafqatullah et al. (2013)
189	0.01	–	–	–	–	262.84	Barua et al. (2015)
–	14.48 ± 0.72	–	–	65.41 ± 0.76	–	–	Vidhani et al. (2016)
2830	400	–	–	–	–	–	Wisdom et al. (2016)
–	–	–	–	–	–	–	Koche et al. (2011)
366	30	–	–	450	–	–	Shafqatullah et al. (2013)
–	–	4.8	2.4	–	2.7	–	Pachkore and Dhale (2012)
372.0 ± 7.8	28.8 ± 4.0	–	–	–	–	–	Gowrishankar et al. (2010)
161.8–2.2	–	–	–	–	–	–	Pachkore and Dhale (2012)

High concentration of calcium and phosphorus is presented in%.
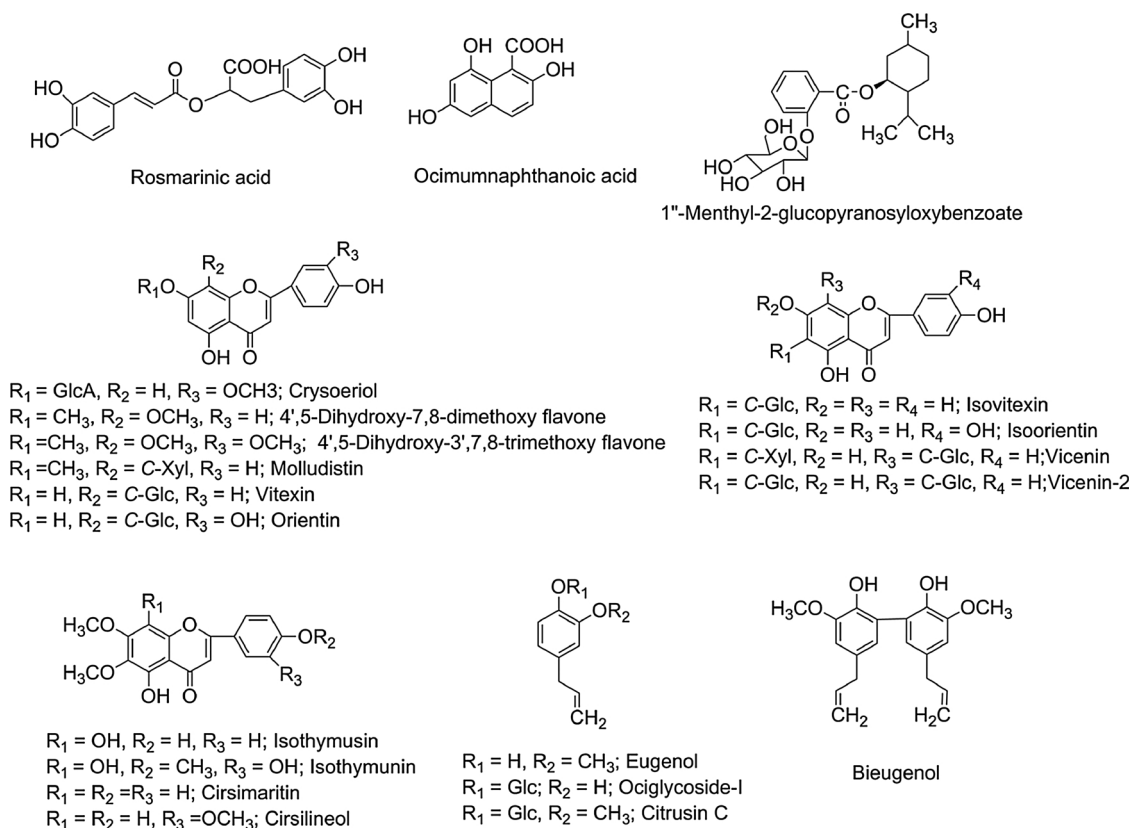
Fig. 1. Structure of major compounds from *O. sanctum*.

oil (18–22%), mucilage, polysaccharides and β-sitosterol in the unsaponifiable matter. *Os* seed oil is rich in triglycerides (94–98%) in which linolenic acid (43.8%) is the main content (Naji-Tabasi and Razavi, 2017). The structure of major secondary metabolites of *Os* is presented in Fig. 1. Table 3 summarizes the list of chemical constituents reported from *Os* and their related biological activities.

4.1. Phenolics

The total phenolic content in *Os* leaves has been found 4.07 ± 0.11 g gallic acid equivalent/100 g dry weight (Koroch et al., 2010). Caffeic acid, chlorogenic acid, vanillic acid, ocimumnaphthanoic acid and menthylsalicylic glucoside were isolated from the aerial parts of *Os* (Skaltsa et al., 1999; Ali and Ali, 2012; Ahmad et al., 2012a). The presence of commonly occurring phenolic compounds gallic acid, gallic acid methyl ester, gallic acid ethyl ester, protocatechuic acid, 4-hydroxybenzoic acid, vanillin and 4-hydroxybezaldehyde were confirmed by HPLC using authentic samples (Norr and Wagner, 1992). Rosmarinic acid, an ester of caffeic acid is quantified as 0.27% *w/w* in *Os* leaves using APCI mass spectrometry technique (Sundaram et al., 2012).

4.2. Flavonoids

Flavonoids are the major class including methoxy flavonoids and their glycosides (luteolin, isothymusin, cirsimartin), *C*-glycosides flavonoids (orientin, isoorientin, isovitexin and vicenin) from *Os* (Kelm et al., 2000, Norr and Wagner, 1992; Skaltsa et al., 1999; Uma Devi and Satyamitra, 2004) (Table 3). Grayer et al., 2001 studied the distribution of 8-oxygenated flavones on *Os* leaf surface using atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and identified apigenin, cirsimaritin, salvigenin, crisilineol, eupatorin, isothymusin and gardenin. The analysis shows that flavone-7-*O*-glycosides are the characteristics of *Os*, whereas luteolin-5-*O*-glucoside considered as the

marker compound in all nine species of *Ocimum*, including *Os* (Grayer et al., 2002). The flavones apigenin, isothymusin, cirsimaritin and crisilineol were isolated from the aerial parts of *Os* (Kelm et al., 2000; Suzuki et al., 2009).

4.3. Phenyl propanoids

Eugenol is one of the most distributed phenyl propanoid in the essential oil of *Os* leaves. Other phenyl propane derivatives such as ociglycoside or eugenyl-β-ᴅ-glucoside, citrusin C, ferulaldehyde, bieugenol and dehydrodieugenol were isolated from the leaves of *Os* (Kelm et al., 2000; Suzuki et al., 2009).

4.4. Neolignans

The methanol extract of *Os* leaves revealed seven novel neolignans named as Tulsinol A to Tulsinol G (Suzuki et al., 2009). These neolignans are formed by the polymerization of eugenol.

4.5. Coumarins

Three coumarins named ocimarin, aeculetin and aesculin were reported from *Os*. (Skaltsa et al., 1999; Gupta et al., 2007).

4.6. Terpenoids

Different terpenoids like sesquiterpenoids (β-caryophyllene and 4,5-epoxy-caryophyllene), abietane diterpenoid (carnosic acid), oleane triterpenoids (oleanolic acid, β-Amyrin-glucopyranoside) and ursane triterpenoids (ursolic acid, urs-12-en-3β,6β,20β-triol-28-oic acid) have been reported from *Os* (Suzuki et al., 2009; Baliga et al., 2013). The quantification studies revealed ursolic acid as the most abundant constituent in *Os* with 0.252%–0.478% *w/w* and 0.62–19.10 mg/g using
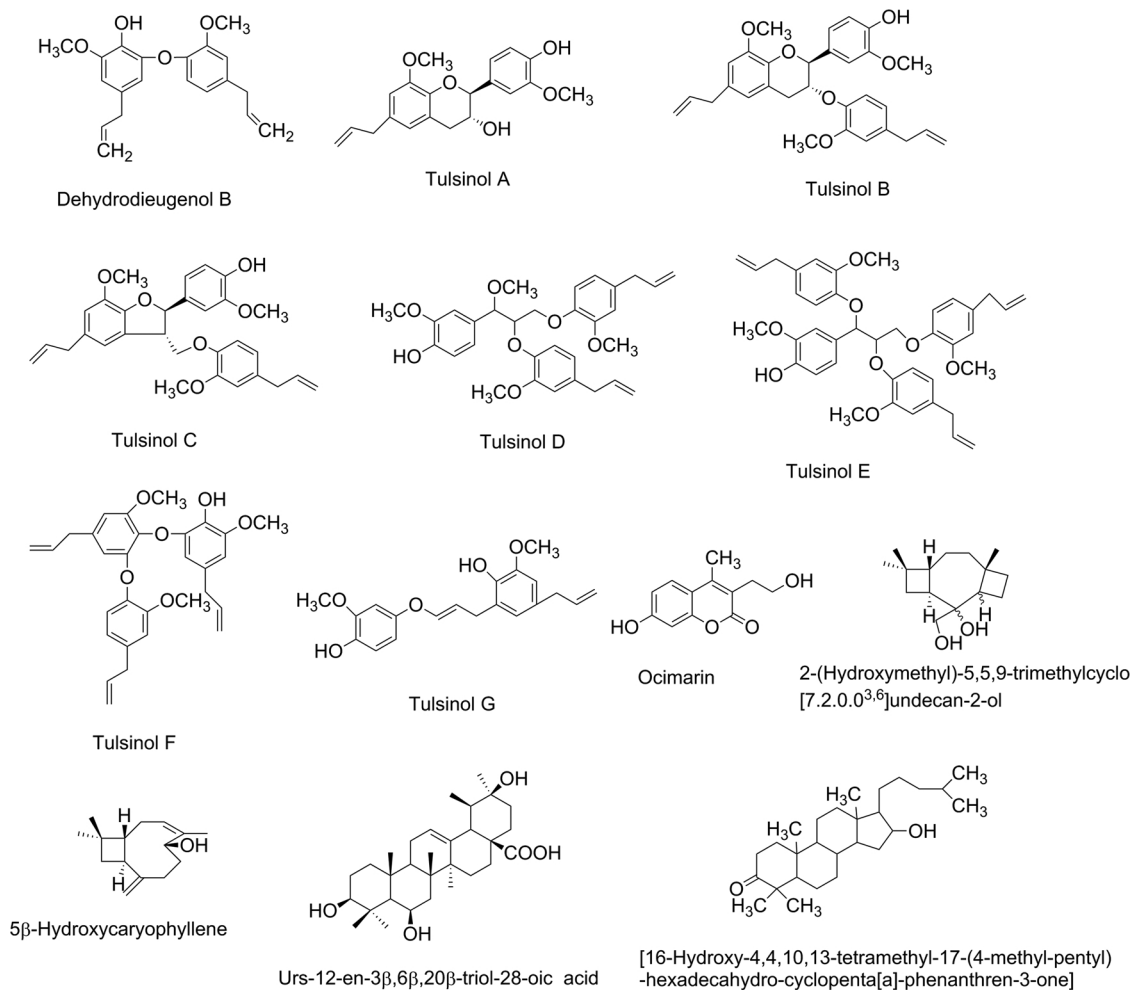
Dehydrodieugenol B

Tulsinol A

Tulsinol B

Tulsinol C

Tulsinol D

Tulsinol E

Tulsinol F

Tulsinol G

Ocimarin

2-(Hydroxymethyl)-5,5,9-trimethylcyclo [7.2.0.0³,⁶]undecan-2-ol

5β-Hydroxycaryophyllene

Urs-12-en-3β,6β,20β-triol-28-oic acid

[16-Hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl) -hexadecahydro-cyclopenta[a]-phenanthren-3-one]

Fig. 1. (*continued*)

HPTLC and UPLC-ESI–MS/MS, respectively (Anandjiwala et al., 2006; Pandey et al., 2014). Two separate antidiabetic activity-guided isolation on *Os* roots and aerial parts provided two novel triterpenoids named urs-12-en-3β,6β,20β-triol-28oic acid and 16-hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl)-hexadecahydrocyclopenta [α] phenanthren-3-one, respectively (Ahmad et al., 2012a; Patil et al., 2011). Further, a new tricyclic sesquiterpenoid 2-(hydroxymethyl)-5,5,9-trimethylcyclo[7.2.0.0³,⁶]undecan-2-ol along with β-caryophyllene, elemene, α-humulene, α-caryophyllene, germacrene-A, trans-α-bergamotene and 5β-hydroxycaryophyllene were isolated from *Os* leaves (Singh et al., 2014). The novel tricyclic sesquiterpenoid was biosynthetically derived from β-caryophyllene (Singh et al., 2014).

4.7. Steroids

Four commonly occurring phytosterols β-sitosterol, β-sitosterol-3-*O*-β-D-glucopyranoside, stigmasterol and campesterol were isolated from leaves and stems of *Os* (Joshi and Karna, 2013; Suzuki et al., 2009; Baliga et al., 2013).

4.8. Essential oil

Os essential oil (yield 0.3-4.1%) is mainly composed of terpenoids including acyclic monoterpenoids, monocyclic terpenoids, bicyclic terpenoids, aliphatic aldehydes, phenolic acids, esters and sesquiterpenoids. The composition and yield of *Os* essential oil are differed with harvesting at different localities, cultivars (green and purple), collection periods, stages of harvesting and climatic conditions

(Saharkhiz et al., 2015; Padalia and Verma, 2011). Eugenol or methyl eugenol and/or methyl chavicol were found as the major constituents of *Os* essential oil by considering the different harvesting stages and cultivars (Mondello et al., 2002; Brophy et al., 1993). The major diversities in *Ocimum species* were found in Africa followed by South America (Brazil) and Asia (India) (Verma et al., 2015). Eugenol (27–83%) was found as the main component of oils from USA, India, Germany, Thailand, Cuba and Brazil, whereas oil from plants grown in Australia contain mainly methyl chavicol (87%) (Brophy et al., 1993; Kicel et al., 2005; Kothari et al., 2005; Vani et al., 2009; Kelm and Nair, 1998). More interestingly, the decreasing concentration of eugenol and methyleugenol contents in *Os* essential oil in matured leaves might be their involvement in polymerization and synthesis of neolignans (Suzuki et al., 2009), and/or further oxidation of phenolic compounds catalyzed by the increase of polyphenoxidase and peroxidase activity (Dey and Choudhuri, 1983). The aroma compounds of *Os* essential oil (methyl eugenol chemotype, 56.18%) were identified by solid phase microextraction (SPME)/GC–MS/flame ionization detection (FID) and olfactoric evaluations. The spicy-green-notes of *Os* essential oil is due to methyl eugenol, β-caryophyllene, β-caryophyllene oxide and germacrene D, while spicy-peppery-notes corresponds to germacrene D (Jirovetz et al., 2003). Moreover, the major pharmacological activities of *Os* essential oil such as mosquitocidal, antimicrobial and anthelmintic were found due to its marker constituent eugenol (Kelm and Nair, 1998; Kumar et al., 2010; Asha et al., 2001). *Os* essential oil (40 μg/ml) was found to be non-toxic to the mammalian kidney fibroblast (VERO) and kidney epithelial cells (LLC-PK11) using Neutral Red assay (Zheliazkov et al., 2008).
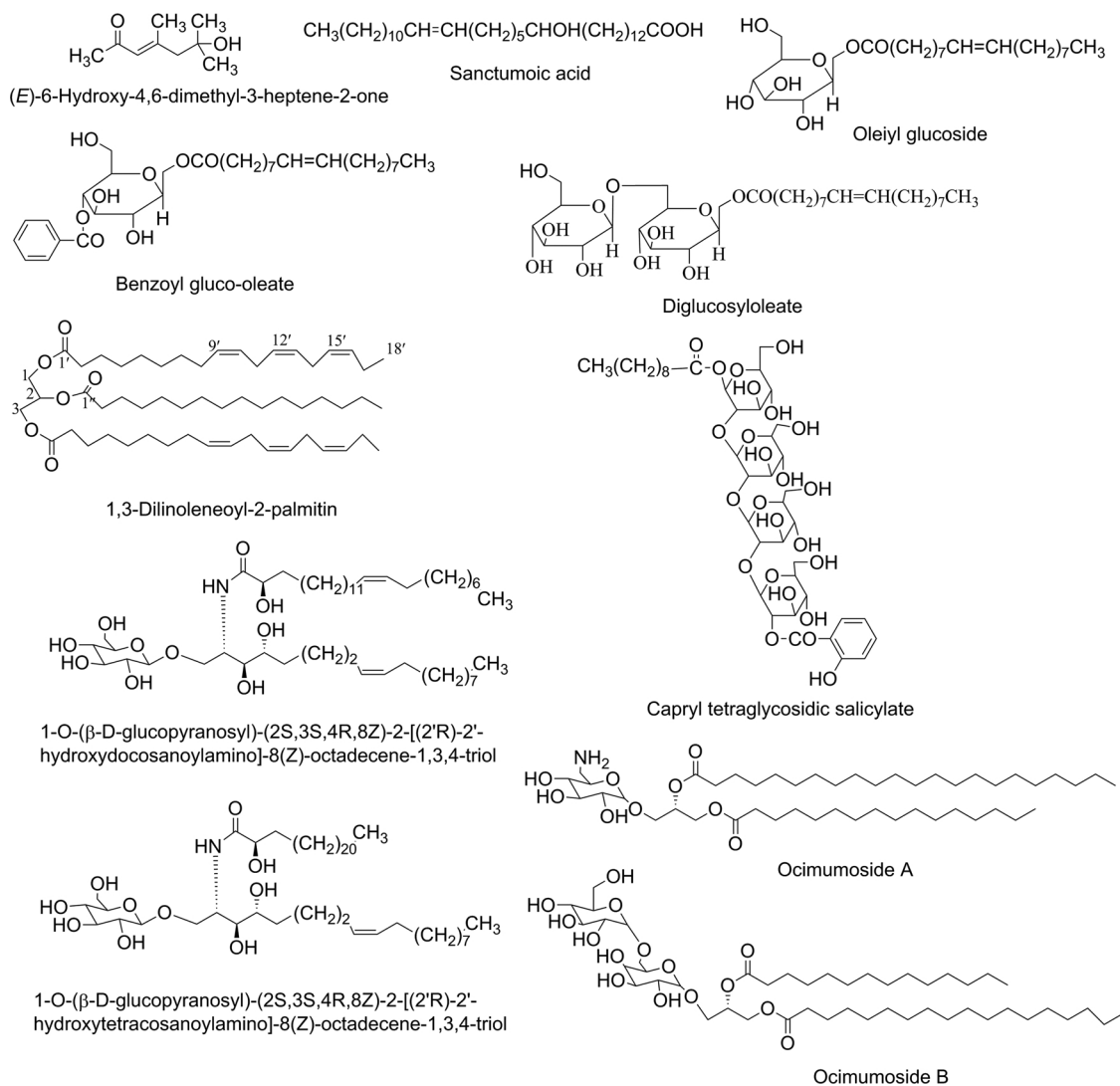
(E)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one

Sanctumoic acid

CH$_3$(CH$_2$)$_{10}$CH=CH(CH$_2$)$_5$CHOH(CH$_2$)$_{12}$COOH

Oleiyl glucoside

Benzoyl gluco-oleate

Diglucosyloleate

1,3-Dilinoleneoyl-2-palmitin

Capryl tetraglycosidic salicylate

1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2'R)-2'-hydroxydocosanoylamino]-8(Z)-octadecene-1,3,4-triol

Ocimumoside A

1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2'R)-2'-hydroxytetracosanoylamino]-8(Z)-octadecene-1,3,4-triol

Ocimumoside B

Fig. 1. (*continued*)

Effect of different cultivars, plant parts, collection period and geographical distribution on the yield of major components of *Os* essential oil obtained by hydrodistillation are discussed in Table 4.

4.9. Fixed oil (non-volatile oil)

The fixed oil content in *Os* seeds was found ∼18–22% and, composed of mainly linoleic acid (66.1%), α-linolenic acid (15.7%), oleic acid (9.0%), palmitic acid (6.94%) and stearic acid (2.1%) (Gupta et al., 2002; Angers et al., 1996; Mondal et al., 2009). The major components of fixed oil, linoleic acid and linolenic acid (an ω-3 fatty acid, *cis*-9,12,15-octadecatrienoic acid) were supposed to be responsible for its anti-inflammatory, anticoagulant, hypotensive, chemopreventive, antihypercholesterolaemic and immunomodulatory activities (Singh et al., 2007). Fixed oil of *Os* is reported for anti-inflammatory, antiarthritic, antimicrobial and antiulcer properties (Singh and Majumdar, 1997; Singh and Majumdar, 1999a; Singh and Majumdar, 1999b; Singh et al., 2001a, 2001b). The anti-inflammatory activity of fixed oil is due to the dual inhibition of arachidonate metabolism and antihistaminic activity (Singh et al., 2007). Only one report is available on the isolation of fixed oil (yield 1.046%) from *Os* leaves, along with the antidiabetic and antioxidant potential. The fixed oil extracted from *Os* leaves was rich in α-linolenic acid (60.60%), linoleic acid (17.86%) and palmitic acid (15.65%) (Suanarunsawat et al., 2016).

4.10. Fatty acid derivatives

Fatty acid derivatives were isolated from the leaves and roots of *Os*, including four cerebrosides (Gupta et al., 2007; Ahmad et al., 2012a). Fatty acid derivatives like palmityl glucoside and sanctumoic acid were exhibited mosquitocidal activity, while cerebrosides showed antistress activity (Kelm and Nair, 1998).

4.11. Polysaccharide

A polysaccharide (∼10^6 Da) isolated from *Os* leaves contain monosaccharide compositions rhamnose (23.3%), xylose (19.2%), arabinose (42.2%), glucose (10.3%) and galactose (5.0%) (Subramanian et al., 2005).

4.12. Other secondary metabolites

An acetone oligomer named (*E*)-6-hydroxy-4,6-dimethyl-3-heptene-2-one was isolated as a colorless oil from the aerial parts of *Os* (Kelm and Nair, 1998).

5. Pharmacological activities of *Os* secondary metabolites

The chemical constituents of *Os* are mainly studied for its

Table 3
Pharmacological activities of secondary metabolites from *O. sanctum*.

Pharmacological activities	Compounds/plant part	Class of compound	Dose(s)	Results	Reference
Anticancer	2-(Hydroxymethyl)-5,5,9-trimethylcyclo [7.2.0.0^{3,6}] undecan-2-ol (Lf)	Sesquiterpenoid	–	IC$_{50}$ 30 ± 0.5 μM against MCF-7 cell line	Singh et al. (2014)
	Luteolin (Lf and Ap)	Flavonoid	50 μM	IC$_{50}$ (78 ± 6 μM) for androgen-independent carcinoma of prostate (LNCaP) and IC$_{50}$ (53 ± 4 μM) for androgen-dependent carcinoma of prostate (PC-3 and DU-145) cells at 72 h.	Nagaprashantha et al. (2011)
	Orientin (Lf and Ap)	Flavonoid	50 μM	IC$_{50}$ (124 ± 7 μM) for androgen-independent carcinoma of prostate (LNCaP) and IC$_{50}$ (104 ± 7 μM) for androgen-dependent carcinoma of prostate (PC-3 and DU-145) cells at 72 h.	Nagaprashantha et al. (2011)
	Vicenin-2 (Apigenin 6,8-diglucoside (Lf and Ap)	Flavonoid	–	IC$_{50}$ (44 ± 3 μM) for androgen-independent carcinoma of prostate (LNCaP) and IC$_{50}$ (25 ± 3 μM) for androgen-dependent (PC-3, DU-145) cells at 72 h.	Nagaprashantha et al. (2011); Nair et al. (1982); Skaltsa et al. (1999)
Antioxidant	Rosmarinic acid (Lf and St)	Phenolic acid	10 μM	Better antioxidant than vitamin E in liposome oxidation model.	Kelm et al. (2000)
	Isothymusin (Lf and St)	Flavonoid	10 μm	Strong antioxidant activity (50% more active than positive control TBHQ and BHT) in liposome oxidation model.	Kelm et al. (2000)
	Isothymonin (Lf and St)	Flavonoid	10 μm	Better antioxidant activity than positive control TBHQ and BHT using liposome oxidation model.	Kelm et al. (2000)
	Cirsimaritin (Lf and St)	Flavonoid	10 μm	Poor antioxidant using liposome oxidation model.	Kelm et al. (2000)
	Cirsilineol (Lf and St)	Flavonoid	10 μm	Good antioxidant activity using liposome oxidation model.	Kelm et al. (2000)
	Eugenol (Lf)	Phenyl propanoid	10 μm	Better antioxidant activity than positive controls TBHQ and BHT using liposome oxidation model.	Kelm et al. (2000)
Anti-inflammatory	Apigenin (Lf, Ap and St)	Flavonoid	1000 μM	Showed 65% COX-1 enzyme inhibition activity, compared to ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	Rosmarinic acid (Lf and St)	Phenolic acid	1000 μM	Showed 58% COX-1 enzyme inhibition activity, compared to ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	Isothymusin (Lf and St)	Flavonoid	1000 μM	Inactive against COX-1 and COX-2 enzyme	Kelm et al. (2000)
	Isothymonin (Lf and St)	Flavonoid	1000 μM	Showed 37% COX-1 enzyme inhibition activity, compared to standard ibuprofen, naproxen and aspirin with 33%, 58% and 46% COX-1 inhibitory activity at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	Cirsimaritin (Lf and St)	Flavonoid	1000 μM	Showed 50% COX-1 enzyme inhibition activity, compared to standard ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	Cirsilineol (Lf and St)	Flavonoid	1000 μM	Showed 37% COX-1 enzyme inhibition activity, compared to standard ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	4′,5-Dihydroxy-3′,7,8-trimethoxy flavone (Lf)	Flavonoid	1000 μM	Showed 37% COX-1 enzyme inhibition activity, compared to standard ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM concentration, respectively.	Kelm et al. (2000)
	Eugenol (Lf)	Phenyl propanoid	1000 μM	Showed 97% COX-1 enzyme inhibition compared to ibuprofen, naproxen and aspirin with 33%, 58% and 46% at 10, 10 and 1000 μM, respectively.	Kelm et al. (2000)

Table 3 (*continued*)

Pharmacological activities	Compounds/plant part	Class of compound	Dose(s)	Results	Reference
Radiation protection	Orientin (Lf and Ap)	Flavonoid	50 µg/kg (i.p.)	Pre-treatment of vicenin protected foetal against irradiation induced genomic damage, and reduced the delayed chromosomal abnormalities and tumorigenesis in pregnant Swiss albino mice.	Uma Devi and Satyamitra (2004)
			6.5, 12.5, 15, 17.5 and 20 µM	Pre-treatment significantly ($p < 0.05$–0.001) reduced the micronucleus counts to 51–67% of RT with dose modification factor (DMF) of 2.62 at 17.5 µM in cultured human peripheral lymphocytes using micronucleus test.	Vrinda and Uma Devi (2001)
			50 µg/kg (*i.p.*)	Pre-treatment showed maximum survival 60% from the 30 days of administration with DMF of 1.30.	Uma Devi et al. (1999)
	Vicenin (Lf)	Flavonoid	50 µg/kg (*i.p.*)	Pre-treatment of vicenin protected foetal against irradiation induced genomic damage, and reduced the delayed chromosomal abnormalities and tumorigenesis in pregnant Swiss albino mice.	Uma Devi and Satyamitra (2004)
			6.5, 12.5, 15, 17.5 and 20 µM	Pre-treatment significantly ($p < 0.05$–0.001) reduced the micronucleus counts to 51–67% of RT with DMF of 2.48 at 17.5 µM in cultured human peripheral lymphocytes using micronucleus test.	Vrinda and Uma Devi (2001)
			50 µg/kg (i.p.)	Pre-treatment showed significant ($p < 0.05$) protection to bone marrow chromosomes. Showed maximum survival 67% from the 30 days of administration with DMF of 1.37.	Uma Devi et al. (1999)
Antidiabetic	16-Hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl)-hexadecahydro-cyclopenta[a]-phenanthren-3-one (Ap)	Triterpenoid	–	Isolated from antidiabetic activity-guided fraction	Patil et al. (2011)
Antistress	Apigenin-7-O-β-D-glucuronic acid (Lf)	Flavonoid	40 mg/kg body weight	Ineffective in Sprague-Dawley rats	Gupta et al. (2007); Norr and Wagner (1992)
	Ociglycoside-I (4-Allyl-1-O-β-D-glucopyranosyl-2-hydroxybenzene/or hydroxychavicol glucoside) (Lf)	Phenyl propanoid	40 mg/kg body weight	Pre-treatment significantly ($p < 0.05$) reduced the increase in corticosterone levels and ($p < 0.01$) reduced the acute stress-induced increase in CK-levels. Less effective on plasma glucose level in acute stress induced Sprague-Dawley rats.	Gupta et al. (2007); Norr and Wagner (1992); Richard et al. (2016)
	7-Hydroxy-3-(2-hydroxyethyl)-4-methyl-2H-1-benzopyran-2-one (ocimarin) (Lf)	Coumarin	40 mg/kg body weight	Ineffective in acute stress male Sprague-Dawley rats	Gupta et al. (2007)
	1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2′R)-2′-hydroxydocosanoylamino]-8(Z)-octadecene-1,3,4-triol (Lf)	Cerebroside	40 mg/kg body weight	Inactive	Gupta et al. (2007)
	1-O-(β-D-glucopyranosyl)-(2S,3S,4R,8Z)-2-[(2′R)-2′-hydroxytetracosanoyla-mino]-8(Z)-octadecene-1,3,4-triol (Lf)	Cerebroside	40 mg/kg body weight	Pretreatment showed antistress activity and reduced significantly ($p < 0.05$) the increase corticosterone levels and effective ($p < 0.01$) reducing the creatine kinase levels in Sprague-Dawley rats.	Gupta et al. (2007)
	(2S)-1-O-hexadecanoyl-2-O-docosanoyl-3-O-[6-deoxy-6-amino-a-D-glucopyranoside]glycerol (Ocimumoside A) (Lf)	Cerebroside	40 mg/kg body weight	Pretreatment showed significant ($p < 0.05$) antistress effects by normalizing hyperglycemia, plasma corticosterone, plasma creatine kinase and adrenal hypertrophy in Sprague-Dawley rats. Ginseng crude powder root, *Panax quinquifolium* (100 mg/kg body weight) was used as standard.	Gupta et al. (2007)
	(2S)-1-O-Octadecanoyl-2-O-tetradecanoyl)-3-O-[a-D-galactopyranosyl-(1″ → 6′)-O-β-D-galactopyranosyl]glycerol (Ocimumoside B) (Lf)	Cerebroside	40 mg/kg body weight	Pretreatment showed antistress activity and reduced significantly ($p < 0.05$) the increase corticosterone levels without affecting plasma glucose level in Sprague-Dawley rats.	Gupta et al. (2007)
Lieshmanicidal	Apigenin ((Lf, Ap and St)	Flavonoid	–	IC$_{50}$ 358.7 µg/ml against *L. major*	

(continued on next page)

Table 3 (*continued*)

Pharmacological activities	Compounds/plant part	Class of compound	Dose(s)	Results	Reference
					Suzuki et al. (2009)
	Luteolin (Lf and Ap)	Flavonoid	–	IC_{50} 73.9 µg/ml against *L. major*	Suzuki et al. (2009); Skaltsa et al. (1999)
	4′,5-Dihydroxy-7,8-dimethoxy flavone (Lf)	Flavonoid	–	IC_{50} > 25 µg/ml against *L. major*	Suzuki et al. (2009)
	4′,5-Dihydroxy-3′,7,8-trimethoxy flavone (Lf)	Flavonoid	–	IC_{50} > 25 µg/ml against *L. major*	Suzuki et al. (2009)
	Ferulaldehyde (Lf)	Phenyl propanoid	–	IC_{50} 0.9 µg/ml against *L. major*	Suzuki et al., (2009)
	Bieugenol (Lf)	Phenyl propanoid	–	IC_{50} 13.6 µg/ml against *L. major*	Suzuki et al. (2009)
	Dehydrodieugenol B (Lf)	Phenyl propanoid	–	IC_{50} 16.9 µg/ml against *L. major*	Suzuki et al. (2009)
	6-Allyl-3′,8-dimethoxy-flavon-3,4′-diol (tulsinol A) (Lf)	Neolignan	–	IC_{50} > 25 µg/ml against *L. major*	Suzuki et al. (2009)
	6-Allyl-3-(4-allyl-2-methoxyphenoxy)-3′,8-dimethoxyflavan-4′-ol (tulsinol B) (Lf)	Neolignan	–	IC_{50} 43.9 µg/ml against *L. major*	Suzuki et al. (2009)
	5-Allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran (tulsinol C) (Lf)	Neolignan	–	IC_{50} 9.1 µg/ml against *L. major*	Suzuki et al. (2009)
	1,2-Bis(4-allyl-2-methoxyphenoxy)-3-(4-hydroxy-3-methoxyphenyl)-3-methoxypropane (tulsinol D) (Lf)	Neolignan	–	Not done	Suzuki et al. (2009)
	1-(4-Hydroxy-3-methoxyphenyl)-1,2,3-tris(4-allyl-2-methoxyphenoxy)propane (tulsinol E) (Lf)	Neolignan	–	IC_{50} 47.1 µg/ml against *L. major*	Suzuki et al. (2009)
	1-Allyl-4-(5-allyl-2-hydroxy-3-methoxyphenoxy)-3-(4-allyl-2-methoxyphenoxy)-5-methoxybenzene (tulsinol F) (Lf)	Neolignan	–	IC_{50} 23.8 µg/ml against *L. major*	Suzuki et al. (2009)
	3-(5-Allyl-2-hydroxy-3-methoxyphenyl)-1-(4-hydroxy-3-methoxyphenoxy)-prop-1-ene (tulsinol G) (Lf)	Neolignan	–	IC_{50} 89.7 µg/ml against *L. major*	Suzuki et al. (2009)
	β-Caryophyllene epoxide (Lf)	Sesquiterpenoid	–	IC_{50} > 25 µg/ml against *L. major*	Suzuki et al. (2009)
	Ursolic acid	Triterpenoid	–	IC_{50} 2.2 µg/ml against *L. major* using amphotericin B (IC_{50} 0.04 µg/ml) as positive control	Suzuki et al. (2009)
	Oleanolic acid	Triterpenoid	–	IC_{50} 17.1 µg/ml against *L. major*	Suzuki et al. (2009)
	β-Sitosterol glucopyranoside (Lf)	Steroid	–	–	Suzuki et al. (2009); Ali and Ali (2012)
	Stigmasterol (Lf)	Steroid	–	IC_{50} > 25 µg/ml against *L. major*	Suzuki et al. 2009
Antimicrobial	Orientin (Lf and Ap)	Flavonoid	400 mg/ml	Active against *S. aureus*, *S. cohni* and *K. pneumonia* with maximum zone inhibition (18.04, 17.13 and 16.11 mm).	Ali and Dixit (2012); Skaltsa et al. (1999)
	Vicenin (Lf)	Flavonoid	400 mg/ml	Effective against *E. coli* and *Proteus* with maximum ZOI (18.84 and 17.16 mm).	Ali and Dixit (2012)

Table 3 (*continued*)

Pharmacological activities	Compounds/plant part	Class of compound	Dose(s)	Results	Reference
Mosquitocidal	Eugenol (Lf)	Phenyl propanoid	100–250 ppm	LD$_{100}$ (200 µg/ml) against fourth-instar *Aedes aegyptii* larvae.	Kelm and Nair (1998)
	(*E*)-6-Hydroxy-4,6-dimethyl-3-heptene-2-one (Lf and St)	Acetone oligomer	–	LD$_{100}$ (6.25 µg/ml) against fourth-instar *Aedes aegyptii* larvae in 24 h.	Kelm and Nair (1998)
	1,3-dilinoleneoyl-2-palmitin (Lf and St)	Triglyceride	–	Inactive against *Aedes aegyptii* larvae.	Kelm and Nair (1998)

Abbreviations: Ap (areal part); Lf (leaf); Rt (root); St (stem); Tw (twig).

therapeutic potential like anticancer, antioxidant, anti-inflammatory, leishmanicidal, radiation protective, mosquitocidal, antimicrobial and antistress activity. The pharmacological activities of *Os* secondary metabolites are discussed here and summarized in Table 3.

5.1. Anticancer activity

A tricyclic sesquiterpenoids 2-(hydroxymethyl)-5,5,9-trimethylcyclo[7.2.0.0^{3,6}]undecan-2-ol isolated from the oil of *Os* leaves showed antiproliferative activity against MCF-7 cell line (IC$_{50}$ 30 ± 0.5 µM) using doxorubicin as standard (IC$_{50}$ 9.7 µg/ml) (Singh and Chaudhuri, 2013; Singh et al., 2014). In continuation of antiproliferative screening against MCF-7 cell line, sesquiterpenes *β*-caryophyllene, 4,5-epoxycaryophyllene and 5*β*-hydroxycaryophyllene showed IC$_{50}$ values 73.0, 7.0 and 4.8 µg/ml, respectively. Compounds rosmarinic acid, apigenin, luteolin, orientin, vicenin-2, ursolic acid and oleanolic acid are well studied for their anticancer potential (Nagaprashantha et al., 2011). The terpenoids and flavonoids are the major class of compounds responsible for the anticancer activity of *Os*.

5.2. Antioxidant activity

Phenolics/flavonoids of *Os* were investigated for their free radical scavenging activity (Kelm et al., 2000). The antioxidant activity guided isolation of *Os* leaves and stems in liposome oxidation model yielded six flavonoids including apigenin, rosmarinic acid, isothymusin, isothymonin, cirsimaritin, cirsilineol along with eugenol (Kelm et al., 2000). Compounds isothymusin, isothymonin and eugenol showed good antioxidant activity at 10 µM, compared to standards TBHQ (ter-butyl hydroquinone) and BHT (butylated hydroxyl toluene). Koroch et al. (2010) found rosmarinic acid as the main constituent responsible for the antioxidant activity of *Os* due to its rapid scavenging effect of free radicals. A polysaccharide (constituted of 23.3% rhamnose; 19.2% xylose; 42.2% arabinose; 10.3% glucose and 5% galactose) isolated from *Os* leaves demonstrated antioxidant activity in DPPH free radical scavenging, anti-lipid peroxidation, hydrogen peroxide scavenging and superoxide radical scavenging assays (Subramanian et al., 2005). The polysaccharide showed potent DPPH free radicals scavenging activity with IC$_{0.2}$ value of 5.61 ± 0.17 µg/ml, compared to α-tocopherol (IC$_{0.2}$ = 11.9 ± 0.2 mM) and BHA (IC$_{0.2}$ = 14.5 ± 2.5 mM). Also, *Os* polysaccharide scavenged ~ 54% and ~79% of superoxide free radicals at 10 and 50 µg/ml, respectively. The antioxidant results showed that *Os* polysaccharide possesses reactive oxygen species scavenging and iron chelating properties. The pretreatment of *Os* polysaccharide at 100 µg/ml protects 30 ± 3.2% mouse splenocytes against γ-ray irradiation. The antioxidant potential of *Os* polysaccharide against oxidative damage to lipid, DNA and splenocytes warrants its application in radiation protection.

5.3. Anti-inflammatory activity

Os leaves and seeds are reported for reducing the level of uric acid,

the causing factor of arthritis and joint inflammation (Sarkar et al., 1990). The anti-inflammatory activity of compounds isolated from *Os* aerial parts named rosmarinic acid, apigenin, isothymusin, isothymonin, cirsimaritin, cirsineol and eugenol has been evaluated for cyclooxygenase-1 (COX-1) and COX-2 inhibitory activities in human prostaglandin H synthase isozymes (hPGHS-1). Eugenol was the most active compound and showed 97% of COX-1 inhibition at 1000 µM, compared to ibuprofen, naproxen and aspirin with 33%, 58% and 46% COX-1 inhibition at 10, 10 and 1000 µM, respectively (Kelm et al., 2000). Moreover, cirsineol, cirsimaritin, isothymonin and apigenin showed 37%, 50%, 37% and 65% COX-1 enzyme inhibition, respectively. Singh et al. (1996) were found that *Os* volatile oil inhibits arachidonic and leukotriene induced inflammation via cyclooxygenase inhibition and lipo-oxygenase pathways in arachidonic acid metabolism. Whereas, *Os* fixed oil exhibited anti-inflammatory effect due to the dual inhibition of arachidonate metabolism and antihistaminic activity (Singh et al., 2007).

5.4. Radiation protective activity

The radioprotective effect of *Os* was first investigated in the aqueous extract of leaves by Uma Devi and Ganasoundari (1995). The optimum dose of extract for radiation protection was found to be 50 mg/kg b.w. (*i.p.*), with LD$_{50}$ 6.0 g/kg body weight. Further, chemical investigation on *Os* aqueous extract gave two water soluble flavonoids orientin (8-*C*-*β*-D-glucopyranosyl-luteolin) and vicenin (8-*C*-*β*-D-xylopyranosyl-8-*C*-*β*-D-glucopyranosyl apigenin). Both, the flavonoids exhibited protective effect against radiation-induced chromosomal damage in mice due to their free radical scavenging and metal chelating effects (Uma Devi et al., 2000). The iron chelating effect of flavonoids inhibited the formation of thiobarbituric acid reactive substances (TBRAS) that protects lipid peroxidation initiated by iron ion bound to the lipid membrane (Uma Devi et al., 2000). Subsequently, Nayak and Uma Devi (2005) have investigated the optimum dose for flavonoids orientin and vicenin i.e. 50 µg/kg body (in vivo) in radiation protection against bone marrow damage. Both flavonoids showed similar protective effects at doses 50 µg/kg, whereas vicenin at a higher dose (150 mg/kg) showed better bone marrow protection (Uma Devi et al., 1998). Additionally, vicenin showed better survival effects than orientin at 30 days with long lasted protective effects (Uma Devi et al., 1999). Also, the dose modification factor was found to be higher for vicenin (LD$_{50}$ = 1.37) than orientin (LD$_{50}$ = 1.30) and exerted equal protective effects against γ-ray-induced lipid peroxidation in mouse liver. The low and non-toxic concentration of orientin and vicenin showed significant radiation protection to human peripheral lymphocytes (in vitro), suggest their clinical application in cancer radiotherapy as normal tissues protector (Vrinda and Uma Devi, 2001).

5.5. Antihyperlipidaemic and antidiabetic activity

Os leaves have been studied for serum lipid lowering activity in both normal albino rabbits and diabetic rats, the antihyperlipidaemic effect

Table 4

Variation studies of major chemical components of essential oil from *O. sanctum*.

Compounds	Content (%), cultivar (collection stage)	Plant parts	Country of origin	References
Monoterpenoids				
(E)-β-Ocimene	5.8 (green cultivar) and 0.9 (purple cultivar)	Fresh aerial parts	India	Padalia and Verma (2011)
	4.2 (vegetative), 10.6 (full bloom) and 6.6 (seed setting) in green cultivar; 5.0 (vegetative), 6.4 (full bloom) and 4.2 (seed setting) in purple cultivar	Fresh aerial parts (leaves, stem and inflorescence)	India	Padalia et al. (2013)
D-Limonene	3.8 (green cultivar) and 0.6 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. (2002)
	4.39	Dried leaves and stem	India	Khan et al. (2010a, 2010b)
Linalool	21.84	Dried leaves and stem	India	Khan et al. (2010a, 2010b)
1,8-Cineole (eucalyptol)	0.7	Fresh leaves	India	Raju et al. (1999)
	8.9 (pre-flowering), 33.0 (flowering), 32.2 (end of flowering) and 15.3 (fruit bearing)	Dried aerial parts	Poland	Kicel et al. (2005)
	20.78 (vegetative), 19.41 (floral budding) and 20.45 (flowering)	Aerial parts	Iran	Saharkhiz et al. (2015)
Sesquiterpenoids				
α-Caryophyllene (α-humulene)	2.0	Dried stalks and leaves	Cuba	Pino et al. (1998)
	1.7	Fresh leaves	India	Raju et al. (1999)
	5.3 (green cultivar) and 2.1 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. (2002)
	8.1 (flowering)	Fresh leaves	Brazil	Trevisan et al. (2006)
	3.3	Dried leaves and stem	India	Khan et al. (2010a, 2010b)
β-Caryophyllene	5.33	Dried leaves	Northern Australia	Brophy et al. (1993)
	23.1	Dried stalks and leaves	Cuba	Pino et al. (1998)
	9.8	Leaves	Brazil	Machado et al. (1999)
	40.7	Inflorescence	Brazil	Machado et al. (1999)
	31.7	Fresh leaves	India	Raju et al. (1999)
	24.4 (green cultivar) and 10.7 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. (2002)
	16.60	Fresh leaves	Southern India	Jirovetz et al. (2003)
	4.37(full bloom)	Main branch	Southern India	Kothari et al. (2004)
	7.97 (full bloom)	Shoot biomass cut at 30 cm above ground	Southern India	Kothari et al. (2004)
	6.37 (full bloom)	Stem	India	Kothari et al. (2005)
	11.97	Inflorescence	India	Kothari et al. (2005)
	29.4 (flowering)	Fresh leaves	Brazil	Trevisan et al. (2006)
	27.6 (green cultivar) and 17.1 (purple cultivar)	Fresh plant	Northern India	Awasthi and Dixit (2007)
	33	–	India	Dohi et al. (2009)
	11.89	Fresh leaves	India	Kumar et al. (2010)
	7.3 (green cultivar) and 8.4 (purple cultivar)	Fresh aerial parts	India	Padalia and Verma (2011)
	12.6 (vegetative), 9.2 (full bloom), 6.6 (seed setting) in green cultivar; 15.6 (vegetative), 8.0 (full bloom) 7.8 (seed setting) in purple cultivar	Fresh aerial parts (leaves, stem and inflorescence)	India	Padalia et al. (2013)
	5.5	Dried aerial parts	India	Verma et al. (2015)
β-Caryophyllene oxide	3.8	Dried stalks and leaves	Cuba	Pino et al. (1998)
	0.0	Leaves and inflorescence	Brazil	Machado et al. (1999)
	18.5	Leaves and inflorescence	Brazil	Machado et al. (1999)
	1.5	Fresh leaves	India	Raju et al. (1999)
	5.1 (green cultivar) and 1.1 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. (2002)
	1.10	Fresh leaves	Southern India	Jirovetz et al. (2003)
	0.75 (full bloom)	Leaves	India	Kothari et al. (2005)
	1.70	Stem	India	Kothari et al. (2005)
	3.02	Inflorescence	India	Kothari et al. (2005)
	2.7	Fresh aerial parts	Nigeria	Gbolade and Lockwood (2008)
	7.5	Dried aerial parts	India	Verma et al. (2015)
β-Bisabolene	15.4 (pre-flowering), 14.4 (flowering), 13.0 (end of flowering) and 20.4 (fruit bearing)	Dried leaves	Poland	Kicel et al. (2005)
	20.99 (vegetative), 13.29 (floral budding) and 18.76 (flowering)	Aerial parts	Iran	Saharkhiz et al. (2015)
	4.3	Dried aerial parts	India	Verma et al. (2015)
γ-Elemene	10.47 (vegetative), 7.7 (floral budding) and 7.8 (flowering)	Aerial parts	Iran	Saharkhiz et al. (2015)

Table 4 (*continued*)

Compounds	Content (%), cultivar (collection stage)	Plant parts	Country of origin	References
β-Elemene	18.0	Dried stalks and leaves	Cuba	Pino et al. (1998)
	5.0	Leaves	Brazil	Machado et al. (1999)
	5.8	Inflorescence	Brazil	Machado et al. (1999)
	6.2	Fresh leaves	India	Raju et al. (1999)
	1.73	Fresh leaves	Southern India	Jirovetz et al. (2003)
	2.54 (full bloom)	Main branch	Southern India	Kothari et al. (2004)
	16.3 (green cultivar) and 4.3 (purple cultivar)	Fresh plant	Northern India	Awasthi and Dixit (2007)
	8.8	Fresh aerial parts	Nigeria	Gbolade and Lockwood (2008)
	11.0 (green cultivar) and 10.9 (purple cultivar) (full bloom)	Fresh aerial parts	India	Padalia and Verma (2011)
	10.8 (vegetative), 9.8 (full bloom) and 11.3 (seed setting) in green cultivar; 17.7 (vegetative), 14.3 (full bloom) and 9.6 (seed setting) in purple cultivar	Fresh aerial parts (leaves, stem and inflorescence)	India	Padalia et al. (2013)
Germacrene D	5.10	Fresh leaves	Southern India	Jirovetz et al. (2003)
	9.14	Fresh leaves	India	Kumar et al. (2010)
	0.1 (green cultivar) and 1.4 (purple cultivar)	Fresh plant	Northern India	Awasthi and Dixit (2007)
	2.4 (green cultivar) and 2.2 (purple cultivar) (blooming)	Fresh aerial parts	India	Padalia and Verma (2011)
9-*epi*-(E)-Caryophyllene	23.68	Fresh leaves	Thailand	Suanarunsawat et al. (2016)
Selinene	< 0.1 (vegetative), 0.1 (full bloom) and 0.1 (seed setting) in green cultivar; 12.3 (vegetative), 9.4 (full bloom) and 9.7 (seed setting) in purple cultivar	Fresh aerial parts (leaves, stem and inflorescence)	India	Padalia et al. (2013)
Phenylpropanoids				
Eugenol	34.3	Dried stalks and leaves	Nigeria	Pino et al. (1998)
	79	Leaves	Brazil	Machado et al. (1999)
	17.6	Inflorescence	Brazil	Machado et al. (1999)
	53.4	Fresh leaves	India	Raju et al. (1999)
	41.7 (green cultivar) and 77.5 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. (2002)
	1.66	Fresh leaves	Southern India	Jirovetz et al. (2003)
	7.28 (main branch), 8.48 (secondary branch) and 4.36 (shoot biomass cut at 30 cm above ground) (full bloom)	Branches	India	Kothari et al. (2004)
	0.79 (full bloom)	Leaves	India	Kothari et al. (2005)
	8.9 (pre-flowering), 4.1 (flowering), 4.6 (end of flowering) and 7.1 (fruit bearing)	Dried aerial parts	Poland	Kicel et al. (2005)
	59.4 (flowering)	Fresh leaves	Brazil	Trevisan et al. (2006)
	46.2 (green cultivar) and 0.6 (purple cultivar)	Fresh plant	Northern India	Awasthi and Dixit (2007)
	0.4	Fresh aerial parts	Nigeria	Gbolade and Lockwood (2008)
	59	–	India	Dohi et al. (2009)
	61.30	Fresh leaves	India	Kumar et al. (2010)
	18.25	Fresh leaves	Thailand	Suanarunsawat et al. (2016)
	67.4 (green cultivar) and 72.8 (purple cultivar) (full bloom)	Fresh aerial parts	India	Padalia and Verma (2011)
	62.2 (vegetative), 64.1 (full bloom) and 58.3 (seed setting) in green cultivar; 35.3 (vegetative), 45.7 (full bloom) and 44.9 (seed setting) in purple cultivar	Fresh aerial parts (leaves, stem and inflorescence)	India	Padalia et al. (2013)
	10.66	–	Thailand	Sutaphanit and Chitprasert, (2014)
	41.70 (green cultivar) and 77.50 (purple cultivar)	–	India	Pandey et al. (2014)
	15.70 (vegetative), 37.15 (floral budding) and 24.63 (flowering)	Aerial parts	Iran	Saharkhiz et al. (2015)
Methyl eugenol	traces (green cultivar) and 0.1 (purple cultivar)	Leaves and inflorescence	Bangladesh	Mondello et al. 2002
	56.18	Fresh leaves	Southern India	Jirovetz et al. (2003)
	75.03 (full bloom)	Main branch	Southern India	Kothari et al. (2004)
	75.25 (full bloom)	Leaves	India	Kothari et al. (2005)
	83.70	Stem		
	65.20 (full bloom)	Inflorescences	India	Kothari et al. (2005)
	Not detected (green cultivar) and 67.8 (purple cultivar)	Fresh plant	Northern India	Awasthi and Dixit (2007)
	44.7	Fresh aerial parts	Nigeria	Gbolade and Lockwood (2008)
	47.06	Fresh leaves	Thailand	Suanarunsawat et al. (2016)
	42.58	–	Thailand	Sutaphanit and Chitprasert (2014)
	50.9	Dried aerial parts	India	Verma et al. (2015)
Methyl chavicol (estragole)	44.63	Dried leaves and stem	India	Khan et al. (2010a, 2010b)
	11.49 (vegetative), 10.61 (floral budding) and 11.40 (flowering)	Aerial parts	Iran	Saharkhiz et al. (2015)
	1.9 (pre-flowering), 12.5 (flowering), 10.2 (end of flowering) and 5.2 (fruit bearing)	Dried leaves	Poland	Kicel et al. (2005)
	87.69	Dried leaves	Northern Australia	Brophy et al. (1993)

is mainly due to its essential oil content (Suanarunsawat et al., 2016). *Os* essential oil riched with eugenol (18.25%), methyl eugenol (47.06%) and β-caryophyllene (23.68%) has been reported to suppress the serum total cholesterol (93.62 ± 3.29) mg/dl and triglycerides (36.29 ± 3.33 mg/dl) in hypercholesterolaemic rats, compared to the negative control i.e. high cholesterol treated rats (total cholesterol, 138.12 ± 10.21 mg/dl and triglyceride, 50.79 ± 2.86 mg/dl). *Os* essential oil also showed antihyperlipidaemic effects comparable with standard drug simvastatin (total cholesterol, 90.35 ± 5.70 mg/dl and triglyceride, 48.50 ± 4.35 mg/dl). The antihyperlipidaemic activity of *Os* essential oil was due to the suppression of liver lipid synthesis, and the presence of phenylpropanoid constituents. These antihyperlipidaemic results suggest that *Os* essential oil is potentially beneficial in the prevention and treatment of diseases like atherosclerosis and cardiovascular disorders (Suanarunsawat et al., 2009).

The fixed oil obtained from fresh *Os* leaves constitute of mainly α-linolenic acid (60.60%), which significantly lower the diabetically-elevated blood glucose levels and serum lipid profile with an increase in serum insulin levels in streptozotocin-induced type 1 diabetes mellitus rats within three weeks (Suanarunsawat et al., 2016). Fixed oil prevents renal injury caused by diabetes mellitus and significantly increases the serum insulin (4.50 ± 0.24 μU/ml) and lower the serum lipid profile (total cholesterol, 70.0 ± 4 mg/dl; triglyceride 45.0 ± 8), compared to the untreated group (serum insulin, 3.22 ± 0.18 μU/ml; total cholesterol, 93.0 ± 7 mg/dl and triglyceride 92.0 ± 7). Fixed oil decreases the levels of creatinine and blood urea nitrogen ($p < 0.001$) 1.27 ± 0.18 mg/dl and 20.2 ± 0.7 mg/dl, respectively. Additionally, fixed oil suppresses the elevated TBRAS level and increases the activity of antioxidative enzymes in the liver and cardiac tissues. Further, exploration of fixed oil potential in the type 2 diabetes mellitus is recommended.

A tetracyclic triterpenoid, 16-hydroxy-4,4,10,13-tetramethyl-17-(4-methyl-pentyl)-hexadecahydro-cyclopenta [a]-phenanthren-3-one isolated from the antidiabetic activity guided fraction of hydro alcoholic extract of *Os* aerial parts (Patil et al., 2011). The bioactive fraction (20 mg/kg) exhibited significant ($p < 0.001$) anti-diabetic activity and decreases the level of serum glucose, triglycerides, LDL cholesterol and total cholesterol in alloxan induced diabetic rats.

5.6. Antistress activity

Os is well known for its adaptogenic and immunomodulatory properties since ancient time and these potentials credited to its antistress activity. The designed extract named *OciBest* obtained by the blending of water and methanol extracts of *Os* whole plant with the required level of active constituents, ociglycoside-I (> 0.1%w/w), rosmarinic acid (> 0.2%w/w), oleanolic acid and ursolic acid (> 2.5%), which was found to be effective against chronic variable stress (Richard et al., 2016). The antistress effects were studied on cortisol release and CHHR1 receptor activity using cell-based assay, while 11β-hydroxysteroid dehydrogenase type-1 (11β-HSD1) and catechol-*O*-methyltransferase (COMT) for cell-free assays. Further, *OciBest* showed inhibitory activity on COMT (IC$_{50}$ = 11.65 μg/ml) and 11β-HSD1 (99.96% at 200 μg/ml) compared to the standard 3,5-dinitrocatechol (IC$_{50}$ = 24.91 nM) and carbenoxolone (61.44% at 600 nM). Moreover, *Os* (6.25–100 μg/ml) also inhibits cortisol release in forskolin-induced human adreno-carcinoma cells (NCI-H295R) and this effect might be attributed by ursolic acid (625 μM to 10 μM) (Sembulingam et al., 1997; Richard et al., 2016). Thus, inhibition of cortisol release, blocking the CRHR1 receptor, inhibition of 11β-HSD1 and COMT effects are found to be responsible for the antistress activity of *Os* (Richard et al., 2016).

In a separate study, the ethanol extract of *Os* leaves and its *n*-butanol fraction significantly ($p < 0.05$) normalize the acute stress and chronic unpredictable stress at a dose of 200 mg/kg body weight, compared to the standard drug *Panax quinquifolium* at a dose of 100 mg/kg body

weight (Gupta et al., 2007). Further, the antistress activity guided isolation of *n*-butanol fraction led to yield three compounds ociglycoside-I, ocimumoside A and ocimumoside B (Gupta et al., 2007). The prior treatment of all three compounds significantly reduces the increased cortisone levels ($p < 0.05$) and creatine kinase levels ($p < 0.01$) at a dose of 40 mg/kg body weight in acute stress induced rats, compared to the normal group. Among all these compounds, ocimumoside A showed potent antistress effects by normalizing hyperglycemia, plasma corticosterone, plasma creatine kinase and adrenal hypertrophy (Gupta et al., 2007). Ociglycoside-I and ocimumoside B were found to be effective against normalizing stress parameters, while ineffective on plasma glucose levels.

The extended study on antistress potential of ocimumoside A and B on chronic unpredictable stress (CUS) normalizes the stress-induced responses like alterations in monoaminergic (nor-adrenalin, dopamine, serotonin and their metabolites like dihydroxyphenylacetic acid, homovanillic acid and 5-hydroxyindole acetic acid), antioxidant systems and changes in plasma corticosterone levels (Ahmad et al., 2012b). Pretreatment of ocimumoside A and B (40 mg/kg body weight *p.o.*) in CUS-induced animals significantly reduce the plasma corticosterone levels 211.50 ± 13.67 and 225.13 ± 13.28, respectively. These results were efficacious with standard antioxidant, melatonin (211.57 ± 117.73 at 20 mg/kg *i.p.*) and CUS-induced animals as control (308.11 ± 24.59). Additionally, ocimumoside A and B normalizes the CUS-induced perturbations of enzymatic activities such as glutathione level and lipid peroxidation in seven days. Interestingly, these compounds do not show any alterations in the baseline values of stress related parameters, when administered alone. Further, the researchers warrant establishing the pathway to modulate the neurotransmitter levels by these compounds. These findings suggest the traditional application of *Os* in adaptogen with modern pharmacological activities. The antistress potential of *Os* compounds shows its application in the treatment of stress-induced neurological disorders and suggest for future studies.

5.7. Lieshmanicidal activity

The essential oil from *Os* exhibited leishmanicidal activity against *Leishmania donovani* (Zheliazkov et al., 2008). The essential oil demonstrated leishmanicidal activity with IC$_{50}$ = 37.3 ± 4.6 μg/ml and IC$_{90}$ = 90.0 ± 4.6 μg/ml, using pentamidine (IC$_{50}$ = 1.46 ± 0.51 μg/ml and IC$_{90}$ = 4.98 ± 1.1 μg/ml) and amphotericin B (IC$_{50}$ = 0.09 ± 0.01 μg/ml and IC$_{90}$ = 0.35 ± 0.12 μg/ml) as positive controls. Interestingly, the major contents of essential oil eugenol and methyl chavicol did not possess leishmanicidal activity, while minor constituent (+)-δ-cadinene (yield 0.168 ± 0.0194%) showed potent leishmanicidal activity (IC$_{50}$ = 4.0 μg/ml and IC$_{90}$ = 7.0 μg/ml).

The hydroalcoholic extract of *Os* leaves inhibits the growth of promastigotes of *L. amazonensis* by 8.8 ± 1.2% at 50 μg/ml and 10.3 ± 1.3% at 100 μg/ml, compared to pentamidine 96.9 ± 0.2% and 99.2 ± 0.3% at 50 and 100 μg/ml (Garcia et al., 2010). The leishmanicidal activity guided isolation of *Os* ethyl acetate fraction resulted ferulaldehyde and ursolic acid with IC$_{50}$ values 0.9 μg/ml and 2.2 μg/ml, respectively against promastigotes of *L. major* compared to positive control amphotericin B (IC$_{50}$ = 0.04 μg/ml) (Suzuki et al., 2009). Eugenol and caryophyllene oxide showed IC$_{50}$ values > 25 μg/ml against *L. major*, while eugenol dimers bieugenol (IC$_{50}$ = 13.6 μg/ml) and dehydrodieugenol (IC$_{50}$ = 16.9 μg/ml) were found better leishmanicidal components. Also, a novel neolignan tulsinol C (5-Allyl-3-(4-allyl-2-methoxyphenoxymethyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran) exhibited potent leishmanicidal activity with IC$_{50}$ value 9.1 μg/ml against *L. major* (Suzuki et al., 2009).

5.8. Antimicrobial activity

Os flavonoids orientin and vicenin were screened against bacterial

strains causing urinary tract infection in human e.g. *Staphylococcus aureus*, *Staphylococcus cohni* (gram positive), and *Escherichia coli*, *Proteus* and *Klebsialla pneumonia* (gram negative) using disc diffusion method (Ali and Dixit, 2012). Orientin (400 mg/ml) showed antibacterial activity against *S. aureus, S. cohni* and *K. pneumonia* with maximum zone inhibition (ZOI) of 18.04, 17.13 and 16.11 mm, respectively. While, vicenin at 400 mg/ml was found to be active against *E. coli* (ZOI, 18.84 mm) and *Proteus* (ZOI, 17.16 mm). Moreover, the synergistic effect of orientin and vicenin (in a ratio of 1:1) on antibacterial activity showed better results in all the strains than individual flavonoids with maximum ZOI of 20.12, 20.75, 20.95 and 20.31 mm at 400 mg/ml concentrations against *E. coli*, *Proteus*, *S. aureus, S. cohni* and *K. pneumonia*, respectively. The antibacterial activity results were concluded that the potent synergistic effect of flavonoids orientin and vicenin can be used as a new choice for the treatment of bacterial infected UTI infections. Further, the use of positive control during antibacterial screening is recommended to validate these results.

5.9. Mosquitocidal activity

The mosquitocidal activity against *Aedes aegyptii* larvae guided fraction of *Os* yielded two compounds eugenol and (*E*)-6-hydroxy-4,6-dimethyl-3-heptene-2-one (Kelm and Nair, 1998). Eugenol and (*E*)-6-hydroxy-4,6-dimethyl-3-heptene-2-one demonstrated mosquitocidal activity with LD_{100} values 200 μg/ml and 6.25 μg/ml, respectively in 24 h, while there was no mortality for control larvae. Further, the researchers suggest to investigate the different *Os* extract in search of novel mosquitocidal compounds.

6. Conclusions and future prospects

Os has been studied for the bio-assay guided isolation of chemical constituents as well as in search of novel molecules from different extracts. Several chemical class of compounds including phenolics, flavonoids, phenylpropanoids, neolignans, terpenoids, coumarins, fatty acid derivatives, essential oil and fixed oil have been reported from this herb. The essential oil of *Os* is a good source of natural eugenol and well explored in analytical, chemical and biological aspects due to its high commercial importance in pharmaceutical, cosmetics and food industry. Fixed oil of the seeds is rich in ω-3 fatty acids and is the recent interest of the research, due to its wide range of pharmacological properties especially in cardioprotection. Flavonoids are the major class of compounds isolated from *Os* and have been found as the main active constituents. The water-soluble flavonoids, orientin and vicenin have been well explored in terms of their radiation protective effects at lower and higher doses. The hydrophilic character of both the flavonoids makes them useful for their antioxidant effect in detoxification as well as radiation protector in cancer therapy. The traditional importance of *Os* as immunomodulator herb was further supported by the isolation of antistress molecules, ocimumoside A and ocimumoside B, which suggest the application of *Os* in the treatment of neurological disorders. Further studies are needed to explore the molecular and cellular mechanism of antistress activity of ocimumoside A and ocimumoside B. So far, the research work carried out on *Os* is mainly focused on the biological activity of its different extracts and essential oil. The literature study showed that eugenol, ursolic acid, rosmarinic acid, orientin, vicenin, ocimumoside A and ocimumoside B are the main active chemical constituents of *Os* and suggesting an opportunity of finding new bioactive molecules.

However, there are several aspects that needed to explore and investigate further: (1) work on the large scale of plant material to isolate sufficient amount of major as well as minor chemical constituents to explore their pharmacological activities and mechanism for therapeutic potential; (2) explore pre-clinically studied compounds for clinical practices, especially in antistress and radiation protection; (3) limited work has been carried out for the isolation and biological studies on the root extracts of *Os*; (4) more than 60 compounds have been isolated, whereas only a few have been explored for pharmacological activities and pre-clinical studies. Overall, there is a need to further research on the chemical aspects of *Os* to get novel molecules with new pharmacological potential. The long history of traditional uses, wide spectrum of pharmacological properties and toxicity studies suggested *Os* as a safe valuable herb for clinical applications. The present compilation of chemical constituents along with their pharmacological properties will be helpful in future studies on *Os* plant as well as in search of new leads for drug discovery.

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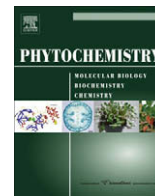
Review

Chemistry and pharmacology of *Rhaponticum carthamoides*: A review

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A B S T R A C T

Rhaponticum carthamoides (Willd.) Iljin is a perennial herb, commonly known as a maral root or Russian leuzea, which has been used for centuries in eastern parts of Russia for its marked medicinal properties. This review based on 117 literary sources, with many of them being originally published in non-English languages (mainly in Russian), discusses the current knowledge of traditional uses, chemistry, biological effects and toxicity of this species. Several different classes of compounds were previously isolated from various parts of *R. carthamoides* of which the main groups are steroids, particularly ecdysteroids, and phenolics (flavonoids and phenolic acids) accompanied with polyacetylenes, sesquiterpene lactones, triterpenoid glycosides and terpenes (essential oil). A comprehensive account of the chemical constituents is given in this review (figures of 120 structures are shown). Various types of preparations, extracts and individual compounds derived from this species have been found to possess a broad spectrum of pharmacological effects on several organs such as the brain, blood, cardiovascular and nervous systems as well as on different biochemical processes and physiological functions including proteosynthesis, work capacity, reproduction, and sexual function. Moreover, the extracts and preparations from the plant, which are hopefully safe, exhibited various additional biological effects e.g. antioxidant, immunomodulatory, anticancerogenic, antimicrobial, antiparasitic and insect antifeedant or repellent activities. The results of data analysis on the chemical, pharmacological and toxicological characteristics of *R. carthamoides* support the view that this species has beneficial therapeutic properties and indicate its potential as an effective adaptogenic herbal remedy. Finally, some suggestions for further research on chemical and pharmacological properties are given in this review.

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Contents

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1. Introduction

Rhaponticum carthamoides (Willd.) Iljin (family Asteraceae), commonly known as a maral root or Russian leuzea, is a perennial herb, up to 150 cm high (Figs. 1 and 2), endemic in the Altai and Saian Mountains of South Siberia, where it naturally occurs in the alpine and subalpine meadows at 1200–2300 m above sea level (Selivanova, 1979; Lotocka and Geszprych, 2004). During the last few decades, the plant has been introduced to various regions of Central and Eastern Europe, where it is now widely grown for its marked medicinal properties (Opletal et al., 1997). The history of *R. carthamoides* as a medicinal plant began ages ago when local hunters in Altai observed the behaviour of the maral deer (*Cervus elaphus sibiricus*), which seemed to restore its strength after feeding on its roots. Their observation gave the traditional name "maral root" to the plant and initiated its use by local healers (Hlava and Valicek, 1989). In traditional medicine of Siberia, it has long been used in cases of overstrain and common weakness after illness (Petkov et al., 1984). In the last century, the muscle- and strength-building qualities of *R. carthamoides* have been thoroughly investigated in Russia, and various preparations have been commonly used by elite Soviet and Russian athletes in order to upgrade psychological and physical reserves which were exhausted by hard training (Gadzhieva et al., 1995). Currently, the extracts or some compounds from roots and rhizomes are used for their adaptogenic and tonic properties in various dietary supplements or nutraceutical preparations to promote muscle growth, treat impotency, eliminate physical weakness and mental weariness, as well as for recovery after surgery, infectious disease or chemical intoxication. They are also included in the formulas of various non-alcoholic beverages, cosmetic and bath products. Dried underground or aerial parts are included in herbal teas (Opletal and Opletalova, 1990; Opletal et al., 1997).

From a botanical point of view, it should be noted that the nomenclature of the genus is particularly confusing because many species of other genera are synonymous (Klein, 2004). Various species of the genera *Centaurea*, *Cnicus*, *Fornicium*, *Leuzea*, *Serratula*, and *Stemmacantha* are commonly listed in plant databases as synonyms for *R. carthamoides* (The International Plant Names Index, 2004). A particular question is whether to employ the name *Leuzea carthamoides* DC. or *R. carthamoides*. A recent treatment of the Cardueae tribe has chosen the latter (Greuter, 2003), suggesting that *L. carthamoides* be an additional synonym, which is, however, still widely used in many pharmacological and phytochemical studies. Nevertheless, despite many previous attempts to elucidate systematics of the genus (e.g. Dittrich, 1973; Holub, 1973, 1974; Soskov, 1978) including the latest one by Greuter (2003), the taxonomical status of the *R. carthamoides* appears to be still unclear and needs more detailed consideration.

2. Chemical composition

Several different classes of compounds were previously isolated from various parts of *R. carthamoides*, with the main groups being steroids, particularly ecdysteroids, and phenolics (Lamer-Zarawska et al., 1996; Opletal et al., 1997).

One of the earlier phytochemical reports regarding ecdysteroids of *R. carthamoides* revealed the isolation of 20-hydroxyecdysone (20E), known previously as β-ecdysone, ecdysterone or polypodine A (**1**), and inokosterone (**12**) from its underground parts (Krasnov et al., 1977). Further investigations identified 20E as the most abundant ecdysteroid in various parts of the plant with a content of 0.04–0.81%, 0.03–1.22% and 0.27–1.51% of dry matter for roots, aerial part and seeds, respectively (Yakubova and Sakharova, 1980; Varga et al., 1986; Opletal and Opletalova, 1990; Repcak et al., 1994; Timofeev et al., 1998). During more than 30 years of intensive research on the chemistry of *R. carthamoides*, 50 various ecdysteroid compounds (Table 1) have been detected in roots, aerial parts or seeds of the plant (Baltaev and Abubakirov, 1988; Girault et al., 1988; Baltaev, 1992a,b, 1995; Pis et al., 1994; Baltaev et al., 1997; Ramazanov et al., 1997a,b; Sadykov et al., 1997; Borovikova and Baltaev, 1999; Borovikova et al., 1999; Vokac et al., 2002; Budesinky et al., 2008). Several sterols, such as β-sitosterol, stigmasterol, Δ7-avenasterol, campesterol, and cholesterol have been detected in the roots (Khomova et al., 1995) and cholesterol, stigmasterol, β-sitosterol, and β-sitostanol in seeds of the plant (Stransky et al., 1998). The structures of ecdysteroids, shown as Fig. 3, were verified using The Ecdysone Handbook (Lafont et al., 2002).

Regarding *R. carthamoides* phenolic compounds, several authors reported the presence of various flavonoids or anthocyanins (Table 2, Fig. 4) in the roots, aerial parts and inflorescences of the plant (Vereskovskii and Chekalinskaya, 1979; Vereskovskii, 1980a,b; Dombi et al., 1989; Varga et al., 1990; Faizieva et al., 1999; Sharaf et al., 2001; Miliauskas et al., 2005; Koleckar et al., 2008a,b). Hajdu et al. (1998) isolated (*E*)-3,3'-dimethoxy-4,4'-dihydroxystilbene (**108**), a substance biogenetically closely related to flavonoids, from the roots of the plant. Besides the flavonoids, a number of phenolic acids (Vereskovskii and Chekalinskaya, 1978; Skiba and Werglarz, 1999, 2003), several lignans (Harmatha and Dinan, 2003; Harmatha et al., 2007), such as carthamogenin (**103**), carthamoside (**104**), trachelogenin (**105**), or tracheloside (**106**), and tannins e.g. ellagic acid (**107**) have also been detected in both underground and aerial parts of the species. Recently, the serotonin phenylpropanoids, namely *N*-(*Z*)-feruoylserotonin (**99**), *N*-(*Z*)-isoferuoylserotonin (**100**), *N*-(*E*)-feruoylserotonin (**101**), and *N*-(*E*)-isoferuoylserotonin
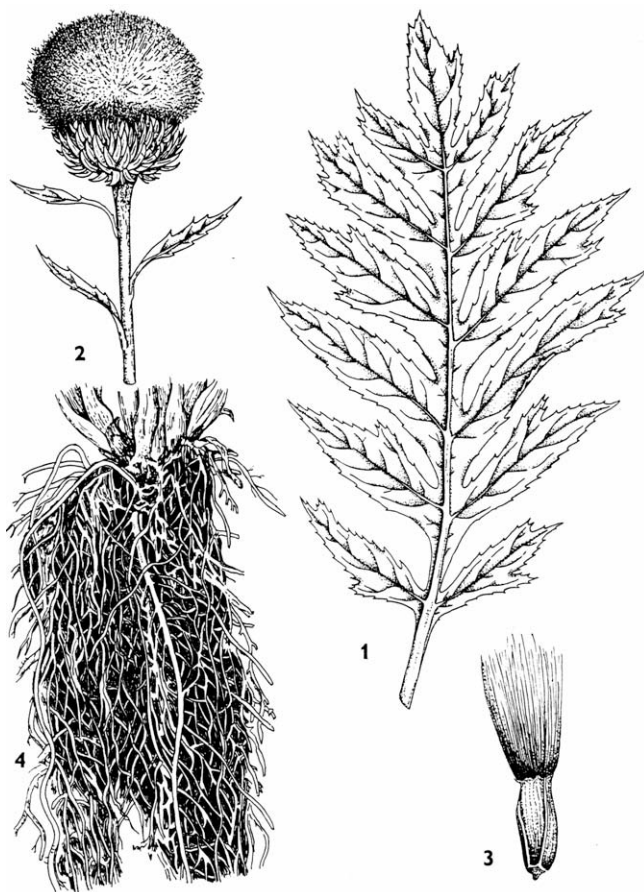

Fig. 1. Line drawing of *R. carthamoides*: 1. leaf, 2. inflorescence, 3. fruit, 4. roots (Valicek et al., 2001).

Fig. 2. *R. carthamoides* plants in flower (Original photo by M. Grbavcic).

(**102**), have been isolated from the seeds (Pavlik et al., 2002; Harmatha et al., 2007). The structures of phenolic acids (Table 3) and other phenol compounds of *R. carthamoides* are shown as Fig. 5.

In regard to other groups of compounds found in *R. carthamoides*, various polyacetylenes (**109–113**) and sesquiterpene lactones as cynaropicrin (**114**), repdiolide (**115**), chlorojanerin (**116**), repensolide (cebellin E) (**117**), and janerin (**118**) have been isolated from roots of the plant (Szendrei et al., 1984; Nowak, 1992; Chobot et al., 2003). Vereskovskii et al. (1978) have found triterpenoid glycosides, namely rhaponticosides A, B, C, D, E, F, G and H in the roots and aerial parts of *R. carthamoides*. Triterpenoid components reported from the literature for underground parts of the plant are parkeol (**119**) and parkeyl acetate (**120**), known also as carthamenol and carthamenyl acetate, respectively (Grimshaw et al., 1981; Khalid et al., 1989). Fig. 6 includes various structures of the miscellaneous compounds mentioned above.

Although essential oil is sometimes mentioned among chemical components of *R. carthamoides*, there are only few reports dealing with its detailed analysis. In one of the earlier reports, Yankulov (1962) observed the presence of essential oil in underground organs with a concentration of up to 0.2%. Recently, Geszprych and Weglarz (2002) reported a content of essential oil in dried plant material ranging from 0.07% to 0.11% and from 0.08% to 0.09% in underground organs and leaves, respectively. The monoterpenes, predominated by geraniol (17.04–18.27%), were the most abundant in the essential oil from roots and rhizomes, whereas sesquiterpenes, represented by β-caryophyllene (24.65–32.30%), was identified as the most important compound in the leaves analysed in their study. A relatively high percentage of linalool (8.88–12.07%) in rhizomes and roots and neeral (8.12–10.22%) in leaves was also observed. In contrast to previous reports, the results of our recently published analysis of the essential oil obtained

Table 1
Ecdysteroid compounds of R. carthamoides.

Structure number	Compound name	Plant parts	References
1	20-Hydroxyecdysone	Root, aerial part, seed	Krasnov et al. (1977), Yakubova and Sakharova (1980), Varga et al. (1986), Baltaev and Abubakirov (1988), Girault et al. (1988), Pis et al. (1994), Repcak et al. (1994), Ramazanov et al. (1997a), Timofeev et al. (1998), Budesinky et al. (2008)
2	Polypodine B	Root	Baltaev and Abubakirov (1988), Girault et al. (1988), Pis et al. (1994)
3	Makisterone A	Root	Pis et al. (1994)
4	2-Deoxyecdysterone	Root	Baltaev and Abubakirov (1988)
5	Integristerone A	Root	Baltaev and Abubakirov (1988), Vokac et al. (2002), Budesinky et al. (2008)
6	Integristerone B	Root	Vokac et al. (2002)
7	Taxisterone	Root	Vokac et al. (2002)
8	Ajugasterone C	Root	Szendrei et al. (1988), Pis et al. (1994), Budesinky et al. (2008)
9	α-Ecdysone	Seed	Sadykov et al. (1997), Budesinky et al. (2008)
10	Lesterone	Seed	Borovikova and Baltaev (1999)
11	Rapisterone D	Seed	Baltaev (1995)
12	Inokosterone	Root	Krasnov et al. (1977), Budesinky et al. (2008)
13	Rapisterone	Root	Baltaev and Abubakirov (1988)
14	20-Hydroxyecdysone 2,3;20,22-diacetonide	Root	Pis et al. (1994)
15	20-Hydroxyecdysone 2,3-monoacetonide	Root	Baltaev and Abubakirov (1988)
16	20-Hydroxyecdysone 20,22-monoacetonide	Root	Baltaev and Abubakirov (1988), Pis et al. (1994)
17	22-Oxo-20-hydroxyecdysone	Root	Vokac et al. (2002)
18	24(28)-Dehydromakisterone A	Root, aerial part, seed	Baltaev and Abubakirov (1988), Girault et al. (1988), Ramazanov et al. (1997a), Budesinky et al. (2008)
19	(24Z)-29-Hydroxy-24(28)-dehydromakisterone C	Root	Vokac et al. (2002), Budesinky et al. (2008)
20	Carthamosterone	Root, aerial part	Vokac et al. (2002), Girault et al. (1988)
21	Rubrosterone	Root	Vokac et al. (2002)
22	Dihydrorubrosterone	Root	Vokac et al. (2002)
23	Posterone	Root	Vokac et al. (2002)
24	Isovitexirone	Root	Pis et al. (1994), Vokac et al. (2002)
25	Leuzeasterone	Root	Vokac et al. (2002)
26	Makisterone C	Root, aerial part	Girault et al. (1988), Vokac et al. (2002), Budesinky et al. (2008)
27	Polypodine B 20,22-acetonide	Root	Pis et al. (1994)
28	Rapisterone B	Seed	Baltaev (1992a)
29	Rapisterone C	Seed	Baltaev (1992b)
30	Rapisterone D 20-acetate	Seed	Borovikova et al. (1999)
31	24(24′)[Z]-Dehydroamarasterone B	Seed	Baltaev et al. (1997)
32	Polypodine B-22-benzoate	Seed	Sadykov et al. (1997)
33	Carthamosterone A	Seed	Ramazanov et al. (1997a)
34	Carthamosterone B	Seed	Ramazanov et al. (1997b)
35	Amarasterone A	Root	Budesinky et al. (2008)
36	Carthamoleusterone	Root	Budesinky et al. (2008)
37	24(28)-Dehydroamarasterone B	Root	Budesinky et al. (2008)
38	22-Deoxy-28-hydroxymakisterone C	Root	Budesinky et al. (2008)
39	3-Epi-20-hydroxyecdysone	Root	Vokac et al. (2002)
40	24-Epi-makisterone A	Root	Budesinky et al. (2008)
41	14-Epi-ponasterone A 22-glucoside	Root	Budesinky et al. (2008)
42	5-α-20-Hydroxyecdysone	Root	Vokac et al. (2002)
43	20-Hydroxyecdysone 2-acetate	Root	Budesinky et al. (2008)
44	20-Hydroxyecdysone 3-acetate	Root	Budesinky et al. (2008)
45	1β-Hydroxymakisterone C	Root	Budesinky et al. (2008)
46	26-Hydroxymakisterone C	Root	Budesinky et al. (2008)
47	15-Hydroxyponasterone A	Root	Budesinky et al. (2008)
48	Inokosterone 20,22-acetonide	Root	Budesinky et al. (2008)
49	Integristerone A 20,22-acetonide	Root	Budesinky et al. (2008)
50	Turkesterone	Root	Budesinky et al. (2008)

by steam distillation from *R. carthamoides* fresh roots using a Clevenger-type apparatus, showed a predominant content of sesquiterpenes, whereas 13-norcypera-1(5),11(12)-diene, cyperene and aliphatic compound aplotaxene were identified as three major constituents of the oil (Havlik et al., 2009). These discrepancies suggest that further investigation of the chemical composition of *R. carthamoides* essential oils could provide interesting information regarding the chemistry of this species.

3. Biological activities

Brekhman and Dardymov (1969) classified *R. carthamoides* as an adaptogen, a term currently used by herbalists to refer to a natural herb product which increases the body's resistance to stresses such as trauma, anxiety and bodily fatigue (Winston and Maimes, 2007).

As is summarized below, the experimental pharmacological and clinical investigations carried out during the last 25 years have shown that extract and individual compounds (especially ecdysteroids) isolated from different parts of the plant have specific biological effects indicating it has marked adaptogenic properties including immunostimulation, eliminating free radicals to prevent oxidizing pathology, increasing protein biosynthesis and physical work capacity along with endurance and performance, enhancing cardiovascular functions and mental work capacity. However, since there is a similarity between some of the ascribed properties of the plant and those ascribed to ecdysteroids, reading of additional literature focusing information on general biological properties of the ecdysteroid compounds, such as review of Lafont and Dinan (2003), is advisable in order to obtain a better understanding of the complete pharmacological potential of the plant.
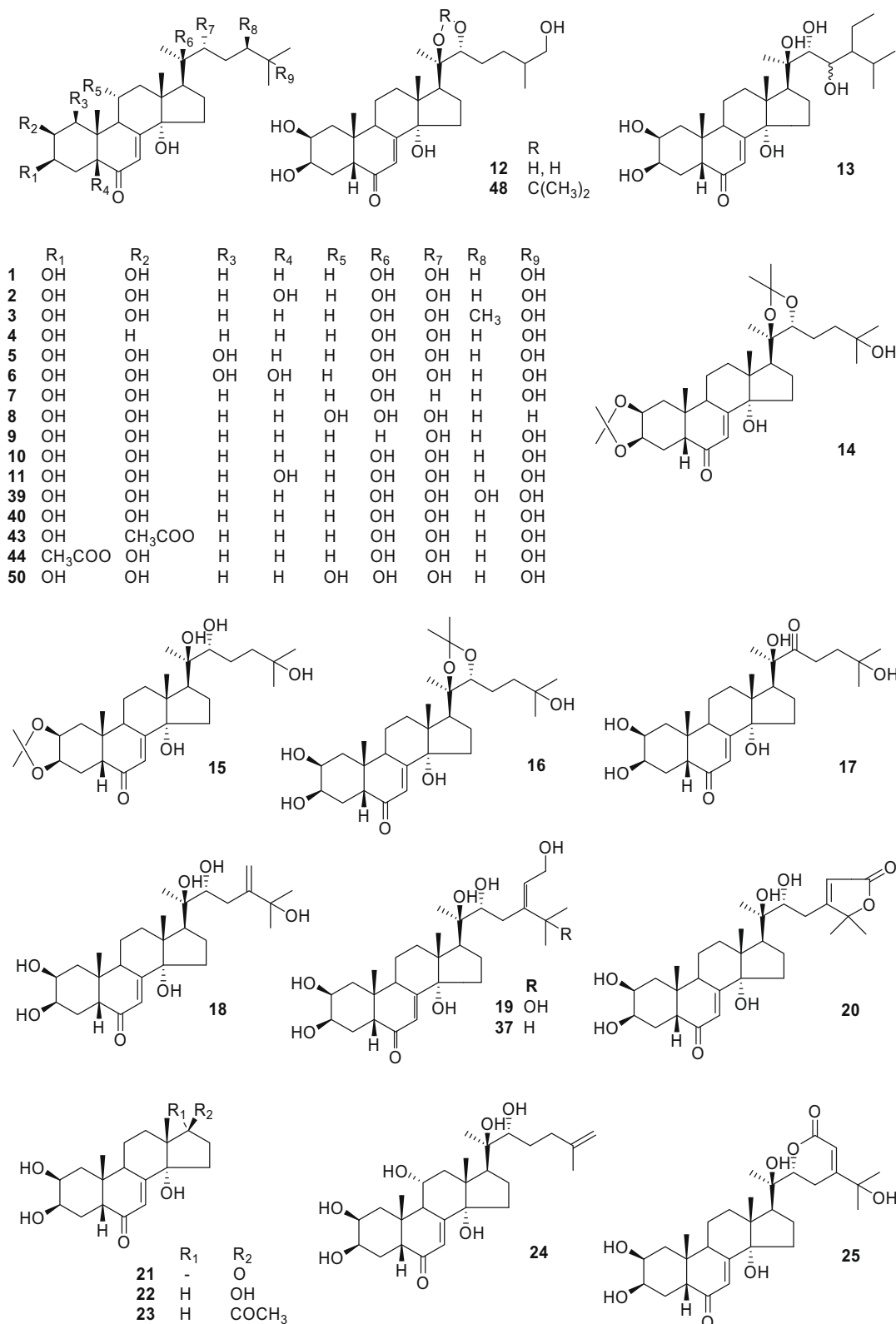
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9
1	OH	OH	H	H	H	OH	OH	H	OH
2	OH	OH	H	OH	H	OH	OH	H	OH
3	OH	OH	H	H	H	OH	OH	CH_3	OH
4	OH	H	H	H	H	OH	OH	H	OH
5	OH	OH	OH	H	H	OH	OH	H	OH
6	OH	OH	OH	OH	H	OH	OH	H	OH
7	OH	OH	H	H	H	OH	H	H	OH
8	OH	OH	H	H	OH	OH	OH	H	H
9	OH	OH	H	H	H	H	OH	H	OH
10	OH	OH	H	H	H	OH	OH	H	OH
11	OH	OH	H	OH	H	OH	OH	H	OH
39	OH	OH	H	H	H	OH	OH	OH	OH
40	OH	OH	H	H	H	OH	OH	H	OH
43	OH	CH_3COO	H	H	H	OH	OH	H	OH
44	CH_3COO	OH	H	H	H	OH	OH	H	OH
50	OH	OH	H	H	OH	OH	OH	H	OH

Fig. 3. The structures of *R. carthamoides* ecdysteroid compounds.

3.1. Effect on proteosynthesis and work capacity

Various *in vivo* tests with animals and clinical tests performed on athletes mainly in the former Soviet Union and Russian Federation countries proved a significant positive effect of *R. carthamoides* extracts and ecdysteroid constituents (especially of 20E) on the increase of protein synthesis and working capacity of tested subjects, subsequently leading to the development of several formulations used to increase strength, endurance and promote muscle growth of athletes (Gadzhieva et al., 1995; Azizov, 1997; Azizov et al., 1997, 1998).
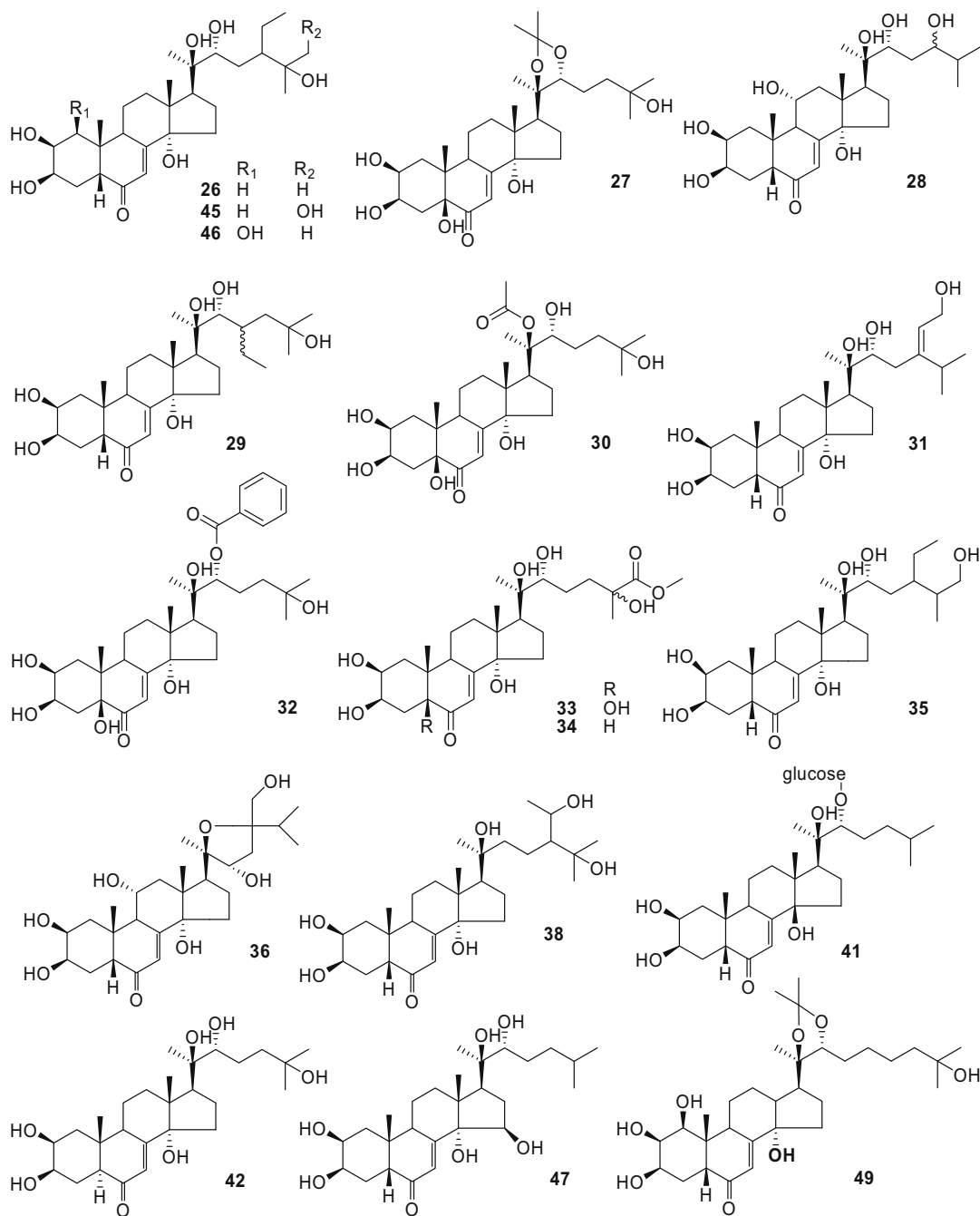
Fig. 3 (*continued*)

Since the 1970s, the anabolic effect of *R. carthamoides* has been reported in a number of experiments on laboratory animals, performed mainly with mice or rats. According to the results of earlier experiments of Syrov and Kurmukov (1976), it is evident that an introduction of *R. carthamoides*-derived 20E at a dose of 5 mg/kg administered to rats for 7 days is shown to be accompanied by an increase in the weight of the liver, heart, kidneys and musculus tibialis anterior. In another study, it was found that 20E isolated from *R. carthamoides* applied daily as intraperitoneal injections to mice, enhanced growth in the groups of female juveniles but not in the male ones; however, it caused increased growth in both males and females adults (Stopka et al., 1999). Growth-promoting effects of *R. carthamoides* derived 20E have also been latterly reported in experiments with Japanese quails (Slama et al., 1996) and pigs (Kratky et al., 1997). A significant increase of the living mass of

freshly hatched Japanese quails was found after 37 days of feeding on a diet containing graded mounts of pulverized seeds of *R. carthamoides* containing 20E (Koudela et al., 1995). In an experiment with male albino mice running on a treadmill, an increase of working capacity (56%) was observed on the 20th day of training after oral administration of *R. carthamoides* extract and a Leveton preparation (consisting of *R. carthamoides* root, bee pollen and vitamins C and E). Similarly, in a swimming endurance test both preparations significantly increased the swimming duration of male albino rats (Azizov and Seifulla, 1998). In more recent studies, Todorov et al. (2000a,b) observed that intraperitoneal injections of aqueous solutions of *R. carthamoides* extract and 20E activates the biosynthesis of macromolecules (protein, RNA, and DNA) in organs of mice.

In clinical trials, preparations named Ecdysten (active ingredient 20E), Leveton and Prime Plus (combination of Ecdysten, unrefined

Table 2
Flavonoids and related compounds of *R. carthamoides*.

Structure number	Compound name	Plant parts	References
51	6-Hydroxykaempferol-7-*O*-(6″-*O*-acetyl-β-ᴅ-glucopyranoside)	Aerial part	Koleckar et al. (2008b)
52	Patuletin	Aerial part	Varga et al. (1990), Koleckar et al. (2008b)
53	6-Hydroxykaempferol-7-glukoside	Aerial part	Varga et al. (1990)
54	Quercetagitrin	Aerial part	Varga et al. (1990)
55	6-Methoxykempferol	Aerial part	Varga et al. (1990)
56	Quercetin-5-glucoside	Aerial part, root	Varga et al. (1990)
57	Isorhamnetin-5-glucoside	Aerial part, root	Varga et al. (1990)
58	Quercetin-3,3′-dimethyl ether	Aerial part, root	Dombi et al. (1989), Varga et al. (1990)
59	Quercetin	Root	Vereskovskii (1980a), Varga et al. (1990), Faizieva et al. (1999)
60	Quercetagetin	Root	Vereskovskii (1980a), Faizieva et al. (1999)
61	Luteolin	Inflorescence, root	Vereskovskii (1980a), Vereskovskii (1980b), Faizieva et al. (1999)
62	Kaempferol	Root	Vereskovskii (1980a), Faizieva et al. (1999)
63	Isorhamnetin	Root	Vereskovskii (1980a), Varga et al. (1990), Faizieva et al. (1999)
64	Quercetin-3-methyl ether	Inflorescence, root	Vereskovskii (1980b), Dombi et al. (1989), Varga et al. (1990)
65	Quercetine-5-*O*-β-D-galactoside	Root	Sharaf et al. (2001)
66	Isorhamnetine 5-*O*-α-L-rhamnoside	Root	Sharaf et al. (2001)
67	Quercetagetin-7-*O*-β-glucopyranoside	Root	Miliauskas et al. (2005)
68	6-Hydroxykaempferol-7-*O*-β-glucopyranoside	Root	Miliauskas et al. (2005)
69	Quercetagetin-7-*O*-(6″-*O*-acetyl-β-glucopyranoside)	Root	Miliauskas et al. (2005)
70	6-Methoxykaempferol-3-*O*-β-glucopyranoside	Root	Miliauskas et al. (2005)
71	6-Hydroxykaempferol-7-*O*-(6″-*O*-acetyl-β-D-glucopyranoside)	Root	Miliauskas et al. (2005)
72	Quercimeritrin	Root	Varga et al. (1990)
73	Apigenin	Inflorescence	Vereskovskii (1980b)
74	Eriodictyol	Aerial part	Koleckar et al. (2008b)
75	Eriodictyol-7-β-glucopyranoside	Aerial part	Koleckar et al. (2008b)
76	Hesperetin	Root	Faizieva et al., 1999
77	Chrysanthemin	Inflorescence, root	Vereskovskii and Chekalinskaya (1979), Faizieva et al. (1999)
78	Cyanin	Inflorescence, root	Vereskovskii and Chekalinskaya (1979), Faizieva et al. (1999)

	R_1	R_2
74	H	H
75	glucopyranose	H
76	H	CH_3

	R
77	H
78	glucose

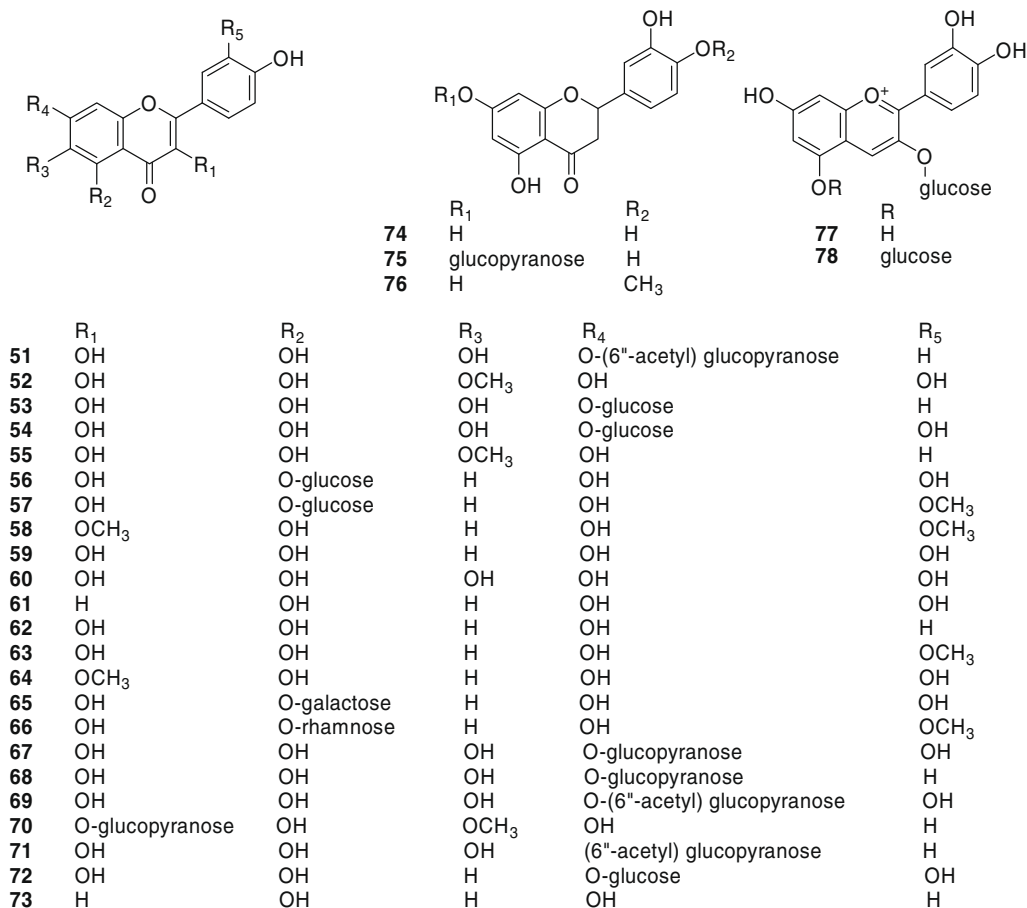|	R_1	R_2	R_3	R_4	R_5
51	OH	OH	OH	O-(6″-acetyl) glucopyranose	H
52	OH	OH	OCH_3	OH	OH
53	OH	OH	OH	O-glucose	H
54	OH	OH	OH	O-glucose	OH
55	OH	OH	OCH_3	OH	H
56	OH	O-glucose	H	OH	OH
57	OH	O-glucose	H	OH	OCH_3
58	OCH_3	OH	H	OH	OCH_3
59	OH	OH	H	OH	OH
60	OH	OH	OH	OH	OH
61	H	OH	H	OH	OH
62	OH	OH	H	OH	H
63	OH	OH	H	OH	OCH_3
64	OCH_3	OH	H	OH	OH
65	OH	O-galactose	H	OH	OH
66	OH	O-rhamnose	H	OH	OCH_3
67	OH	OH	OH	O-glucopyranose	OH
68	OH	OH	OH	O-glucopyranose	H
69	OH	OH	OH	O-(6″-acetyl) glucopyranose	OH
70	O-glucopyranose	OH	OCH_3	OH	H
71	OH	OH	OH	(6″-acetyl) glucopyranose	H
72	OH	OH	H	O-glucose	OH
73	H	OH	H	OH	H

Fig. 4. The structures of flavonoid compounds of *R. carthamoides*.

Table 3
Phenolic acids of *R. carthamoides*.

Structure number	Compound name	References
79	Benzoic acid	Skiba and Weglarz (2003)
80	*m*-Hydroxybenzoic acid	Skiba and Weglarz (2003)
81	*p*-Hydroxybenzoic acid	Skiba and Weglarz (2003)
82	Salicylic acid	Skiba and Weglarz (2003)
83	Protocatechuic acid	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
84	Gentisic acid	Skiba and Weglarz (2003)
85	Vanillic acid	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
86	Gallic acid	Skiba and Weglarz (2003)
87	Syringic acid	Skiba and Weglarz (2003)
88	*o*-Coumaric acid	Skiba and Weglarz (2003)
89	*p*-Coumaric acid	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
90	Caffeic acid	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
91	Ferulic acid	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
92	Sinapic acid	Skiba and Weglarz (2003)
93	*o*-Hydroxyphenylacetic acid	Skiba and Weglarz (2003)
94	*p*-Hydroxyphenylacetic acid	Skiba and Weglarz (2003)
95	Chlorogenic	Vereskovskii and Chekalinskaya (1978), Skiba and Weglarz (2003)
96	Neochlorogenic	Vereskovskii and Chekalinskaya (1978)
97	Isochlorogenic acid a	Vereskovskii and Chekalinskaya (1978)
98	Isochlorogenic acid b	Vereskovskii and Chekalinskaya (1978)

With exception of *o*-coumaric acid detected in aerial part only, all phenolic acids were found in both aerial and underground parts of the plant.
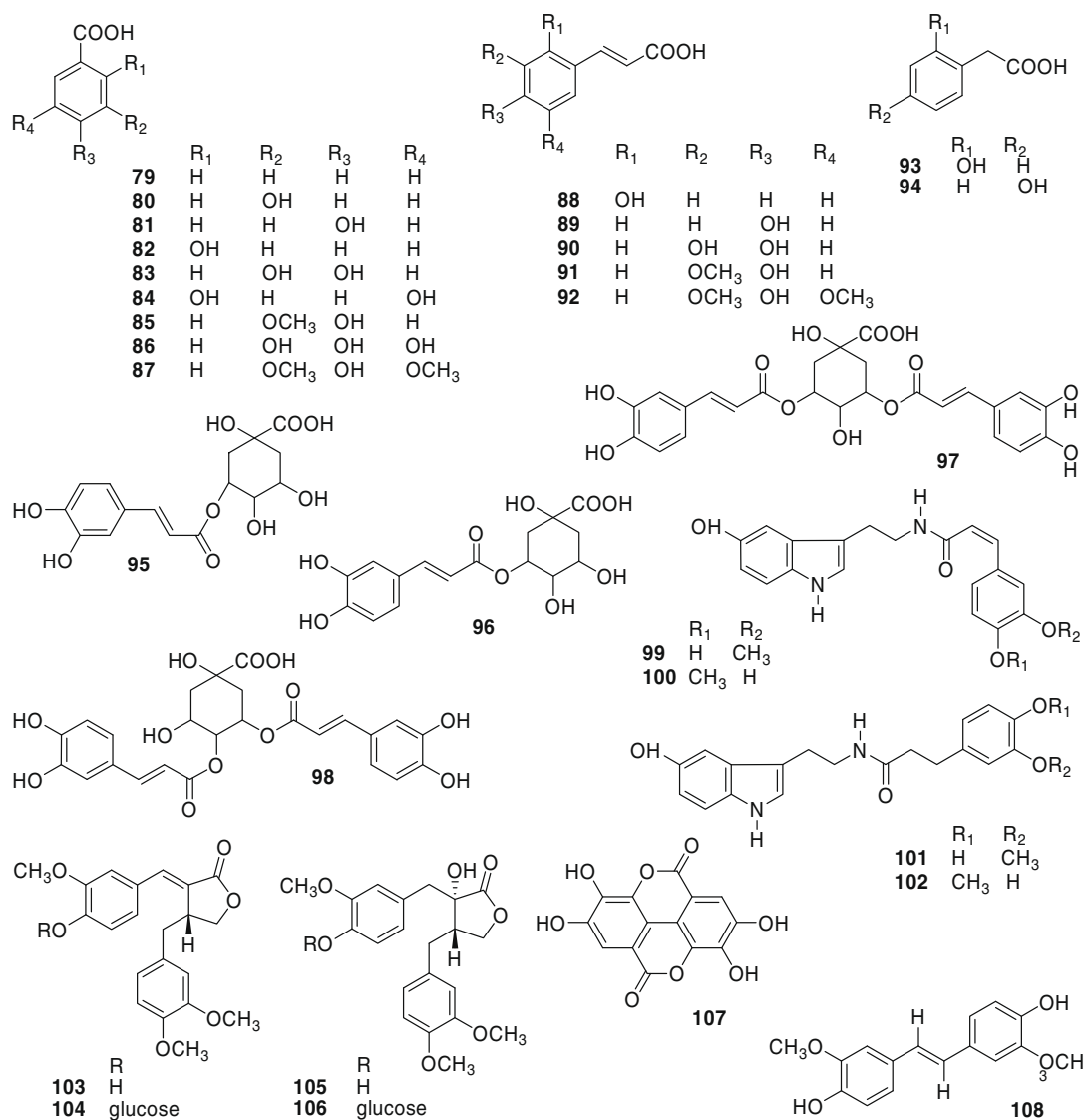

Fig. 5. The structures of phenolic acids, tannins and lignans of *R. carthamoides*.

Fig. 6. Miscellaneous compounds of *R. carthamoides*.

sugar and casein) taken orally for 20 days by three separated groups of track-and-field athletes under conditions of daily aerobic-anaerobic training significantly diminished fat content and elevated the muscle mass in all groups of treated individuals in comparison with the control group (Gadzhieva et al., 1995). Further studies performed over a 20-day administration of *R. carthamoides* tincture and Leveton caused a marked reduction of the blood coagulation potential accompanied with an increase in the working capacity and rehabilitation of top athletes under intensive physical activity (Azizov, 1997). It also possessed an effect on the humoral immunity of track and field runners, whereas treatment with both preparations significantly contributed to restoration of the lowered immunoglobulins A (IgA) and G (IgG), as well as of the complement component 3 concentration in blood serum. Furthermore it increased working capacity by 10% to 15% (Azizov et al., 1997). A subsequent study showed that a 20-day administration of Leveton reduced the level of malonic dialdehyde in the urine of highly skilled athletes and increased their physical working capacity in bicycle ergometry exercise tests with gradually increasing physical loads (Azizov et al., 1998).

3.2. Influence on cardiovascular system and blood

Considering experiments on the cardiovascular system and blood characteristics, Petkov et al. (1984) demonstrated that the dried residue of ethanol (40%) extract re-dissolved in saline and administrated intraduodenally to chloralose-anesthetized male cats in a dose of 200 mg/kg exerted a significant decrease in blood pressure by 25–30% as compared to control group of animals. Per os administration of 20E at a dose of 5 mg/kg markedly stimulated secretion and improved chemical characteristics of bile because of decreased cholesterol content and increased levels of bile acids and bilirubin in normal rats; moreover, it exerts more beneficial parameters when studied in rats with toxic hepatitis induced by heliotrine (Syrov et al., 1986). The root ethanol extract exhibited marked antidiabetic properties by decreasing the blood level of glucose and increasing the reduced level of liver glycogen in experiments on mice and rats with alloxan-induced diabetes (Molokovskii et al., 1989). A dry extract was studied for the haemorheological activity in spontaneous hypertensive rats. A 5-day treatment of the test rats with acute myocardial infarction, given a daily dose of 150 mg/kg, improved rheological characteristics of blood such as reduced viscosity of whole blood and plasma, increased spontaneous erythrocyte aggregation and fibrinogen

concentration and increased erythrocyte deformability and electrophoretic mobility (Plotnikov et al., 1999, 2001). Repeated prophylactic administration of plant adaptogen preparation based on extract from *R. carthamoides* produced a pronounced anti-arrhythmic effect on the model of adrenal arrhythmia in rats (Maimeskulova and Maslov, 2000). A recent study has also demonstrated insulin-stimulating and antihyperglycaemic activities (Molokovskii et al., 2002). Repeated administration of *R. carthamoides* derived 20E increased the content of erythrocytes and haemoglobin in the blood and cause a marked effect on red blood regeneration in haemotoxic phenylhydrazine anaemia in rats (Syrov et al., 1997). A preparation consisting of flavonoids isolated from *R. carthamoides* namely hesperetin, quercetin, quercetagetin, luteolin, kaempferol, isorhamnetin, chrysanthemin and cyanin has been found to prevent the accumulation of cholesterol and triglycerides in triton WR 1339 induced hyperlipidemic rats (Khushbaktova and Syrov, 1989). In a further study, *R. carthamoides* flavonoids substantially diminished the manifestations of an atherosclerotic process in rabbits, whereas their therapeutic effect was associated both with the hypolipidemic activity and the capacity of flavonoids to inhibit local mechanisms of atherogenesis, enhancing vascular wall resistance (Khushbaktova et al., 1991). Recently, *in vitro* anti-platelet activity of crude ethanol extract and flavonoids from aerial parts of *R. carthamoides* was determined in human platelet-rich plasma. Arachidonic acid (AA), adenosine diphosphate (ADP), collagen (COL), and thrombin were used as agonists of platelet aggregation. The summary extract showed a significant inhibition of the aggregation induced by COL and ADP. Of the tested flavonoids, eriodictyol and patuletin influenced COL- and AA-induced aggregation (Koleckar et al., 2008a).

The data from a number of *in vivo* studies summarised above demonstrated a significant positive effect of *R. carthamoides* derived extracts, 20E and flavonoids on the cardiovascular system and blood characteristics in animal models with various induced or spontaneous disorders such as atherosclerosis, anti-arrhythmia, diabetes, myocardial infarction and hepatitis.

3.3. Effect on nervous system and brain

As shown later in this section, several *in vivo* tests with rats or rabbits showed a significant effect of various extracts, ecdysteroids and *N*-feruloylserotonin isomers isolated from *R. carthamoides* on mammalian nervous systems. In one of several studies, the water–ethanol extract from roots injected as a saline solution at

500 mg/kg doses increased the locomotor activity, showed a clear-cut tendency towards antagonizing the narcotic effects of chloral hydrate and towards increasing CNS excitability (jumping test), and also improved the learning and memory indices in rats 60 min before training (Petkov et al., 1984). The root ethanol extract demonstrated a significant improvement in learning and memory ability, as well as completely eliminating the scopolamine-induced memory impairment using the maze-training method of active avoidance with punitive reinforcement in experiments with rats (Mosharrof, 1987). The oral administration of 150 mg/kg of dried *R. carthamoides* root extract prepared by extraction with 40% ethanol to rats with cerebral ischaemia for 5 days prevented destructive changes and decreased the density of synapses in the cerebral cortex (Logvinov et al., 2001). The administration of Ecdysten at doses of 5–10 mg/kg to rabbits resulted in an expressed activation of animal EEG and exerts an awakening effect, reducing natrium thiopental and chloralosa induced hypnosis. This preparation produced an antihypnotic effect by reducing animal sleep in the experiments with mice in combined administration with chloral hydrate and hexenal. Additionally, multiple administrations of Ecdysten to rats stimulated their emotional and research activity, shortening the time of conditioned defence reflex development (Syrov and Khush-baktova, 2005). As recently reported by Yamamotova et al. (2007), the *N*-feruloylserotonins rich fraction isolated from seeds of *R. carthamoides* possessed selective stress-reducing effects in rats with pre-existing tendencies toward anxiety.

In only one study on the nervous system performed with human subjects, a decoction of *R. carthamoides* root (half glass, 4–5 times a day during 2 month) promoted a correction of the depressive manifestation in alcoholics with depressive states, and also improved health conditions of patients with gastrointestinal pain of somatic origin. The decoction was prepared in the following manner: boiling water was poured into a glass containing a tablespoon of pulverized plant material and additionally boiled for 30 min in laboratory water bath, then cooled at room temperature for 10 min, filtered, topped up with water to the full volume of the glass and flavoured with teaspoon of honey (Ibatov, 1995).

3.4. Effect on reproduction and sexual function

The aphrodisiacal reputation of the plant has been studied in several experiments demonstrating the positive influence of 20E on sexual functions of human subjects, but at the same time, showing inconsistent results in tests on animals. For example, Mirzaev et al. (2000) reported that a 10-day administration of 20E (5 and 10 mg/kg) significantly improved the behavioural characteristics of the sexual function of rats, whereas in experiments performed by Stopka et al. (1999), daily peritoneal injections of 20E isolated from *R. carthamoides* inhibited the production of sperm in male and caused disturbances of the oestrous cycles in the female mice. In trials with humans, the administration of Ecdysten to men with an infertility diagnosis (disturbed spermatogenesis as a complication of some urologic diseases) increased the copulative function and improved the sperm quality as well as improved sexual function of patients in the stage of recovery after myocardial infarction (Mirzaev et al., 2000).

3.5. Anti-oxidative, immunomodulatory and anticancerogenic activity

In a great number of *in vitro* tests, Miliauskas et al. (2004) have screened acetone, ethyl acetate and methanol extracts of 12 medicinal and aromatic plants for its radical scavenging activity (RSA) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Among all extracts tested, high RSA was observed for a methanol extract from leaves and stems of *R. carthamoides* in both assays. The high radical scavenging ability was evidently caused by a high content of polyphenolic compounds such as flavonoids and flavonols. A hyphenated LC-DAD-SPE-NMR setup in combination with on-line radical scavenging detection has subsequently been applied for the identification of radical scavenging compounds of *R. carthamoides* aerial parts. The analysis led to the discovery of a new compound 6-hydroxykaempferol-7-*O*-(6″-*O*-acetyl-β-D-glucopyranoside), however the results of the DPPH test showed that its radical scavenging activity was weaker than that of the reference antioxidants rosmarinic acid and Trolox (Miliauskas et al., 2005). In a study performed by Biskup and Eojkowska (2006), aqueous and methanol leaf extracts exhibited RSA towards DPPH (IC_{50} = 25 and 45 µg/ml, respectively) and turned out to be more effective than BHT (IC_{50} = 190 µg/ml), but less effective than ascorbic acid and α-tocopherol (IC_{50} = 4 and 12 µg/ml, respectively). In a recent comparative study, Koleckar et al. (2008b) assayed 88 extracts from various parts of plants from European Asteraceae and Cichoriaceae for radical scavenging activity by means of DPPH. Among all samples tested the extract from the leaves of *R. carthamoides* (IC_{50} = 0.046 mg/ml) was chosen as the most promising material for a subsequent phytochemical study, which resulted in isolation of seven different compounds. Their antioxidant activity was evaluated by DPPH and ferric reducing antioxidant power (FRAP) tests and compared with Trolox and quercetin. Both tests evaluated 6-hydroxykaempferol-7-*O*-(6″-*O*-acetyl-β-D-glucopyranoside) as the most active antioxidant.

The polysaccharide-rich fraction from the fresh leaves markedly enhanced phytohemagglutinin-induced proliferation of human lymphocytes up to a concentration of 150 µg/ml and significantly decreased the release of oxygen free radicals by human granulocytes *in vitro* indicating that the plant contains constituents endowed with immunomodulatory activity (Lamer-Zarawska et al., 1996). In a recent paper by Harmatha et al. (2008) discussing the lack of immunostimulatory activity of ecdysteroids, namely 20E, polypodine B, ajugasterone C, inokosterone, makisterone A, carthamosterone, poststerone, rubrosterone and dihydrorubrosterone, the authors suggest that classes of substances other than ecdysteroids, e.g. lignans, flavonoids or sesquiterpene lactones are more likely responsible for the immunopharmacological effect of the plant.

Hamburger et al. (2006) recently reported that lipophilic root extract exhibited effects on the proliferation of human breast cancer cell line MCF-7, whereas 20E was found to be inactive. In another study, chloroform, methanol, and aqueous leaf extract, as well as pure 20E, exhibited no, or only mild, cytotoxic activity against HeLa (cervical carcinoma) and HL-60 (leukaemia) cell lines, using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Biskup and Eojkowska, 2006).

One of the rare *in vitro* studies addressing the effect on immunological characteristics found that the combination of *R. carthamoides* extract and sarcolysin inhibited the process of lympholeucosis in mice (Vershinina, 1967). Results of more recent research showed that a preparation based on *R. carthamoides* root extract possessed an inhibitory effect on the carcinogenesis induced by transplacental administration of *N*-nitrosylurea. Yearlong administration of the drug was followed by a higher survival rate of the rats and a lower occurrence and/or multiplicity of tumours, mainly those of the central nervous system (Bespalov et al., 1992).

In a human clinical study, a plant preparation called AdMax, a combination of dried ethanol/water extracts from roots of *R. carthamoides*, *Rhodiola rosea*, *Eleutherococcus senticosus* and fruits of *Schizandra chinensis*, was studied by Kormosh et al. (2006) with respect to its influence on the immunity in ovarian cancer patients. Twenty eight patients with stage III–IV epithelial ovarian cancer were treated once with 75 mg/m^2 cisplatin and 600 mg/m^2 cyclophosphamide. Peripheral blood was collected 4 weeks after

chemotherapy. Subclasses of T, B and NK lymphocytes as well as immunoglobulin M (IgM), IgA and IgG concentrations were tested in the blood samples. In patients who took AdMax (270 mg a day) for 4 weeks following chemotherapy, the mean numbers of the four T cell subclasses were increased in comparison to the mean numbers of the T cell subclasses in patients who did not take AdMax. In patients who took AdMax, the mean amounts of IgG and IgM were also increased. The obtained results suggest that the combination of extracts from adaptogenic plants (including *R. carthamoides*) may boost the suppressed immunity in ovarian cancer patients who are subject to chemotherapy.

In summary, the above mentioned data shows that the results of various *in vitro* assays performed with *R. carthamoides* extracts and their fractions or individual compounds, such as lignans or flavonoids, suggest promising antioxidantive, immunomodulary and anticancerogenic properties of the plant, however only few of them have been further investigated using *in vivo* or clinical studies.

3.6. Antimicrobial and antiparasitic activity

The *in vitro* inhibitory effect of various crude extracts from roots or aerial parts of *R. carthamoides* on Gram-positive bacteria, e.g. *Bacillus cereus*, *Staphylococcus epidermidis*, *Bacteroides fragilis* (Kokoska et al., 2002, 2005) and some fungi (Jahodar et al., 2003) have previously been observed. The lipid fraction from seeds has demonstrated antimicrobial activity against *Proteus vulgaris, Aspergillus niger* and *Penicillium verrucosum* (Shirshova et al., 1999). In our experiments, ethyl acetate fraction obtained from *R. carthamoides* aerial part inhibited *in vitro* growth of 19 different strains of *Staphylococcus aureus*, with minimum inhibitory concentrations (MICs) ranging from 128 to 512 µg/ml. This fraction exhibited potent activity against clinical isolates, which showed an associated resistance to oxacillin, ciprofloxacin and erythromycin (Janovska et al., 2008). The thiophene polyene (*E*)-2-[5-(hept-5-en-1,3-diynyl)-thien-2-yl]-ethan-1,2-diol isolated from ethanol extract of underground parts demonstrates significant antifungal activity mainly against *Candida glabrata, Aspergillus fumigatus* and *Trichophyton mentagrophytes* var. *mentagrophytes* with MICs ranging from 4 to 32 µg/ml. The activity after 24 h of incubation against *A. fumigatus* and *Candida tropicalis* was ½ and ¼ of the effect of ketoconazole, respectively (Chobot et al., 2003).

Regarding the anti-infective actions of *R. carthamoides*, there has only been one study performed with human subjects. As a result of this trial, the preparation Ecdysten has shown significant efficacy in the treatment of patients with persistent and acute giardiasis (Osipova et al., 2002).

Since growth inhibitory action of 20E on bacteria and fungi was observed at rather high concentrations ranging between 100 and 400 µg/ml (Ahmad et al., 1996), some other effective group of compounds previously isolated from the plant such as polyacetylenes seems to be responsible for its antimicrobial effect. However, ecdysteroids may also participate in the general anti-infective action of *R. carthamoides*, especially considering its antiparasitic properties.

3.7. Effect on insects

It is currently generally accepted that certain ecdysteroids play crucial roles in the control of insect growth, development, metabolism, metamorphosis, and reproduction. 20E is the principle steroid hormone in insects; however, recent data suggest that other related ecdysteroids can exert similar effects (Simonet et al., 2004). Ecdysteroids are the steroid hormones of all classes of arthropods and most likely of other invertebrates as well. The function of ecdysteroid analogues (phytoecdysteroids) in plants is still

conjectural, but it is believed that they provide some degree of protection against non-adapted phytophagous insects and/or soil nematodes (Dinan, 2001). Despite the well known influence of phytoecdysteroids on insects, the effects of *R. carthamoides* on invertebrate organisms have only been partially studied. Among several researchers working in this area, Pavela (2002) reported strong repellent and antifeedant activities of ethanol extract from seeds on adults of the Colorado potato beetle (*Leptinotarsa decemlineata*). In another study published by the same author (Pavela et al., 2005), two ecdysteroid compounds, namely ajugasterone C and polypodine B decreased the fecundity of cabbage aphids (*Brevicoryne brassicae*) which fed on rape plants contaminated by water solutions of both separately tested substances. The mortality of larvae and adults significantly increased in plants treated with a specific fraction containing 20E, ajugasterone C, polypodine B and at least six other minor constituents, as well as with both individual compounds ajugasterone C and polypodine B. Although 20E has been found to be the best tolerated compound of all phytoecdysteroids tested against cabbage aphids in the study of Pavela et al. (2005), according to the results of Calas et al. (2006), 20E purified from *R. carthamoides* significantly inhibited feeding and oviposition in larvae and adults of the European grapevine moth (*Lobesia botrana*).

In addition to ecdysteroids, two lignan glycosides derived from *R. carthamoides*, namely tracheloside and carthamoside, exhibited different effects on the insects varying from high feeding deterrent activity e.g. tracheloside for the Confused flour beetle (*Tribolium confusum*) larvae, to significant feeding stimulant activity e.g. carthamoside for the Grain weevil beetle (*Sitophilus granarius*) adults in experiments performed by Harmatha and Nawrot (2002). Additionally, several sesquiterpene lactones previously detected in *R. carthamoides* appeared to be good antifeedants, with the most active substance being chlorojanerin (Cis et al., 2006).

In view of the data summarised above, the ecdysteroids are the main compounds of the plant affecting growth, development, and reproduction of insects, whilst its feeding behaviour is also significantly influenced by other groups of *R. carthamoides*-derived constituents, such as lignans and sesquiterpene lactones.

4. Toxicology

Among a number of plant species containing phytoecdysteroids, *R. carthamoides* was shown to be very safe even at high doses. In one of the earliest toxicological studies performed by Petkov et al. (1984), a root water–ethanol extract applied intraperitoneally and subcutaneously in doses up to 40 000 mg/kg did not produce mortality in male albino-mice even 7 days after its application. In the chick embryotoxicity screening test, 20E, and polypodine B isolated from roots of the plant were found not to be embryotoxic (Kosar et al., 1997). The only study showing a certain degree of toxicological risk related to *R. carthamoides* was performed by Chobot et al. (2006) on (*E*)-2-[5-(hept-5-en-1,3-diynyl)-thien-2-yl]-ethan-1,2-diol, the thiophene polyene isolated from roots of the plant. The results showed its apparent phototoxic activity, which was higher in comparison to the standard photosensitizer xanthotoxin, in histidine photo-oxidation and brine shrimp (*Artemia salina*) assays, as well as in test with sludge worms (*Tubifex tubifex*).

5. Conclusions

A great number of pharmacological and phytochemical studies carried out during last 30 years have demonstrated the vast medicinal potential of *R. carthamoides*, especially its marked adaptogenic effect. Various types of preparations, extracts and individual compounds derived from this species have been found to possess

Among several classes of biologically active compounds identified in *R. carthamoides*, ecdysteroids are assumed to be its main active principle, responsible for the majority of the pharmacological effects. However, other components described in this review such as flavonoids, phenolic acids, lignans, polyacetylenes, sesquiterpene lactones, or triterpenoid glycosides may, to some degree, augment the pharmacological effects of the plant.

various pharmacological effects on several organs such as the brain, blood, cardiovascular and nervous systems as well as on different biochemical processes and physiological functions including proteosynthesis, work capacity, reproduction, and sexual function, that together with some other biological actions e.g. antioxidant and immunomodulatory activities, indicates marked adaptogenic properties of the plant. Moreover, some other biological activities including anticancerogenic, antimicrobial, antiparasitic and insect antifeedant or repellent effects have been reported for extracts or individual compounds of *R. carthamoides*.

The data summarised above, together with the low toxicity potential of the plant, strongly support the view that the *R. carthamoides* has beneficial therapeutic properties indicating its potential as an effective adaptogenic herbal remedy. However, further studies are needed to understand the complex pharmacological action and full phytochemical profile of the plant. Clarification of the chemical composition and biological actions of the essential oil, together with verification of inconsistent results regarding reproduction and sexual function, and detailed investigation of the anticancerogenic potential or effects on insect behaviour suggest the most up-to-date challenges for the future research of *R. carthamoides*.

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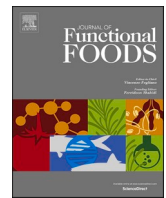
Potential benefits of incorporating *Astragalus membranaceus* into the diet of people undergoing disease treatment: An overview

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ABSTRACT

Astragalus membranaceus (AM) is a notable herb found among medicinal and food plants. Its benefits stem from bioactive compounds that improve function of the immune system, thus preventing and curing many diseases and improve wellness. However, despite of the many potential health benefits, it is mainly used in *Traditional Chinese Medicine* (TCM). Therefore, this review describes its biologically active compounds and the associated health benefits found in recent studies of this herb, particularly with regards to neurodegenerative diseases, diabetes, and cancers along with the way of consumption, doses, and reasons for incorporating it into the diet. AM has positive effects on general health and reduces the risk of neurodegenerative diseases, type 1 and 2 diabetes and cancers development. It also helps improving disease treatments and reduces its side effects associated with food intake. Moreover, this herb has an acceptable organoleptic quality and characteristics that can improve general quality of food. This review is intended to shed light on the many potential benefits of including AM in the diet. However, further studies on specific food recipes for each disease, its effectiveness, as well as its side effects, need to be done to confirm these potential benefits.

1. Introduction

Plants have been consumed for millennia as source of food, medicines, and applied as cosmetics and fragrances. At least 12,000 plants are used as medicinal herbs due to suspected or demonstrated health benefits (IARC, 2002). Among medicinal plants, *Astragalus membranaceus* (AM) is prominent; it is in the top 50 essential herbs used in *Traditional Chinese Medicine* (World Health Organization, 1999). It possesses many pharmaceutical properties; for example, it is an antioxidant, anti-inflammatory, and immunostimulant, all of which help improve general health and help prevent disease, and in some cases, even help treat diseases (Shan, Zheng, & Li, 2019). AM also has an anti-aging property that blocks or slows the aging process in cells and organs (Liu, Zhao, & Luo, 2017). As a pharmaceutical, it is used to protect neurons and prevent inflammation; thus, it has potential for preventing chronic neural degenerative diseases such as *Alzheimer's disease* (AD) and *Parkinson's disease* (PD) (Chan et al., 2009; Singh et al., 2010). AM is also a well-known anti-cancer and anti-diabetes plant, which is attributed to active biological compounds such as *Astragalus polysaccharide* (APS)

(Agyemang et al., 2013; Liu, Yu, et al., 2018; Chen, Bi, et al., 2019). Another remarkable health benefit that has attracted the attention of researchers is its positive effect when integrated into disease treatments. It helps reduce side effects, especially treatments that reduce appetite, e. g., chemotherapy and treatments for cells degenerative diseases and organs failure (Piao & Liang, 2014; Costa et al., 2018; Chen, Bi, et al., 2019). However, despite all of its advantages, the consumption of this herb is not widespread. It is mainly used as a tonic in traditional medicines, often taking the form of common beverages such as teas and tinctures, or as dietary supplements (Percival &Turner, 2007).

One explanation for the limited use of medicinal herbs might be their strong impact on the organoleptic qualities of food (i.e., the taste, smell, color, and texture of food) (Augustin & Sanguansri, 2015). This also explains why many herbal extracts or essential oils are used for things like aromatherapy or by the fragrance industry, or only as a flavoring, i. e., at higher doses, they can strongly alter the sensory appeal of food (Burdock, 1998). However, unlike other herbs, AM has a very mild impact on organoleptic qualities, which makes it much more suitable for use in food (WHO, 1999). With all of its proven health benefits and

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favorable organoleptic characteristics, AM has the potential for daily use in the food of people with the abovementioned health problems. Therefore, this review will examine the advantages and importance of this well-established herbal medicine as food additive that can promote well-being and overall better health and ease some of the appetite-related side effects associated with certain disease treatments.

2. Phytochemistry of *Astragalus membranaceus*

A. membranaceus is one of the most remarkable herbs in the history of medicinal plants, particularly in China. It belongs to the *Fabaceae / Leguminosae* family. It is a native tropical plant, mainly cultivated in Korea, China, and Mongolia (Fu et al., 2014). This plant has a good reputation based on its use as a dried root (*Astragali Radix* or *Huangqi*) in *Traditional Chinese Medicine* (WHO, 1999). It is also listed in the *Chinese* and *Japanese Pharmacopoeia* (Japanese Pharmacopoeia, 2016; Gong et al., 2018). Three published review articles describe the phytochemicals of *A. membranaceus*, summarized the main active compounds include flavonoids, saponins, and polysaccharides. AM contains arounds 40 flavonoids, grouped as flavones, flavanols, isoflavones, isoflavanes, chalcones, and pterocarpans. Isoflavones are the major components in the flavonoids group (with calycosin-7-O-β-D-glucoside as dominant) (Li et al., 2014; Bratkov, Shkondrov, Zdraveva, & Krasteva, 2016). Other reviews of Fu et al. (2014) and Li et al. (2014) summarized the major active compounds saponins. The *Astragalus saponin* group (AST) abounds with more than 80% of *Astragalosides* I (AS-I), II (AS-II), and IV, as well as *isoastragaloside* I and II, which belong to triterpene saponins. *Astragaloside* IV is known as the major active saponin. The chemical structures of these important bioactive compounds (AS-I, AS-IV) and *isoastragaloside* I are presented in Fig. 1 and list of flavonoids and saponins groups are presented in Table 1.

The most important bioactive compounds are *Astragalus polysaccharides* (APS), consisting mainly of glucose, rhamnose, galactose, arabinose, xylose, mannose, glucuronic acid, and galacturonic acid depending on the way of the preparation and purification of APS. The main chain contains linked α-(1 → 4) glucose residues. The relative molecular mass of APS is 5.6×10^3–10^6 Da (Wang, Jia, et al., 2019). Further information about the preparation of APS and their properties are given, e.g. in publications of Chen et al. (2015) and Zheng et al. (2020).

3. *Astragalus membranaceus* potential for health

3.1. Immunoprotecting, anti-inflammation, and anti-aging

A. membranaceus has long been utilized in *Traditional Chinese Medicine*, going back more than 2,000 years (Gong et al., 2018). It is used for many common health problems as well as to improve overall health. Aside from its use as a tonic, there have been several studies conducted both *in vitro* and *in vivo*, with the goal of improving its pharmaceutical and clinical applications. This medicinal root contains many compounds with health and protective properties, e.g., immune stimulants, adaptogens, antioxidants, and anti-inflammatory agents that can prevent and cure diseases (Cui et al., 2018; Liu, Yu, et al., 2018; Shan, Zheng, & Li, 2019).

A. membranaceus and its active compounds have immunomodulating and protective effects that can improve immune organs such as the liver, kidneys, spleen, abdominal organs and tissues and bone marrow (Auyeung, Han, & Ko, 2016; Huang et al., 2019). The mechanism of immune enhancement of APS on macrophages stimulation and regulation via promoting phosphorylation of protein kinase (Akt), nuclear factor-kB (NF-kB), interferon (IRF3), and Ras-Raf-MEK-ERK (MAPKs) pathways in RAW264.7 cells. It is involved in the inhibition of the production of inflammatory factors and their gene expression. Akt and IRF3 are the main immunomodulating pathways by APS to induced macrophage, in which predominantly Akt affects the downstream of the MyD88 dependent and independent pathways of the Toll-like receptor 4 (TLR4) (Li et al., 2017). APS improves immunity in mice via proliferation of B cells from spleens by mediating macrophage activity in TLR4 (Shao et al., 2004). Other mechanism of AM compounds in immune responses involve in stimulation of APS on function T and B lymphocytes, to secrete lymphocyte cytokines such as interferon gamma (IFN-γ) and interleukins (IL-2, IL-4, IL-6, IL-10, IL-12) to improve macrophage and phagocytosis (Liu & LV, 2020). Chen et al. (2020) recently reviewed inclusively about all the immunomodulation and protection mechanisms of *Astragali radix* of AM product on innate immunity, immune organs, mucosal immune, and pharmacological and immunological diseases influenced by its bioactive compounds (flavonoids, saponins, APS). In immune organ, APS is the predominant compound that affects development of immune organs such as spleens, pancreas and liver to fight foreign pathogens. Moreover, it also helps improve mucosal immune system by increasing immunoglobulin A secretion and proliferate intestinal mucosa. In innate immunity, the mechanism of APS and other active compounds increase phagocytic macrophage via increase nitrogen oxide (NO), TNF-α, IL-1, and promote inducible nitric oxide synthase (iNOS). AM also help in maturation and differentiation of cells to

Isoastragaloside I

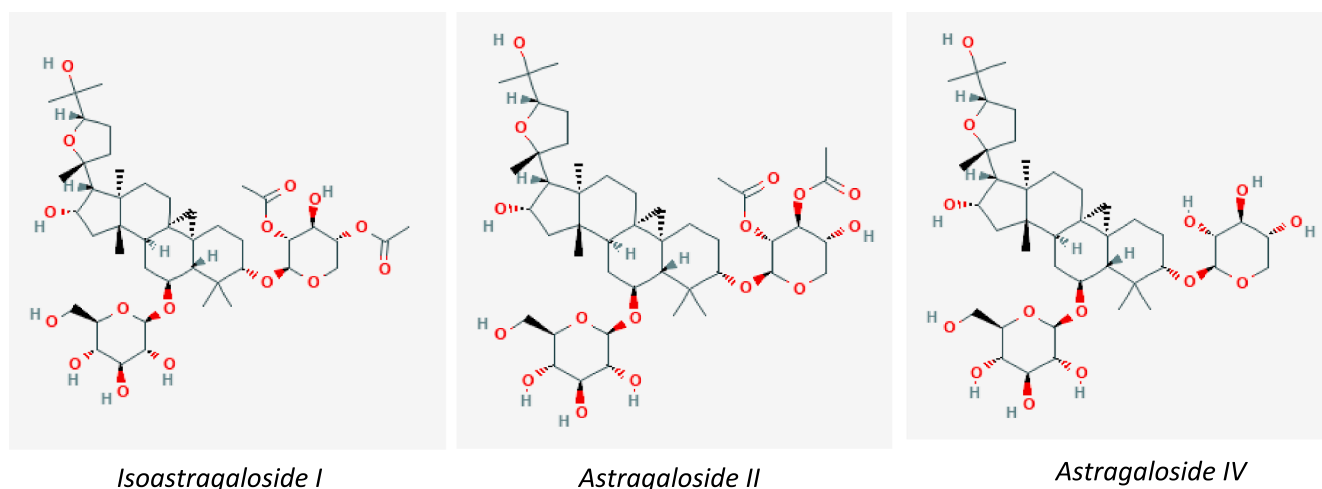Astragaloside II

Astragaloside IV

Fig. 1. Chemical structures of *Isoastragaloside I, Astragaloside II* (AS-II)*, and Astragaloside IV* (AS-IV), extracted from PubChem (2020).

Table 1
Bioactive compounds flavonoids and saponin groups of *Astragalus membranaceus.*

Groups	Compounds	References
Flavonoids	**Flavones**	Li et al. (2014)
	5,6-dihydroxyflavone	Bratkov, Shkondrov,
	7-O-glucuronic acid	Zdraveva, & Krasteva,
	Flavonols	(2016)
	Clovin	Li et al. (2019a)
	Quercetin	Li et al. (2019b)
	Flagaloside C	
	Falkozid C	
	Rhamnocitrin (7-methylkaempferol)	
	Rhamnetin (7-methylquercetin)	
	5-deoxykaempferol	
	Fisetin (5-deoxyquercetin)	
	Kaempferol Isorhamnetin (3,5,7,4′-tetrahydroxy-3′-methoxyflavone)	
	Hyperoside (3-O-β-D-gal and quercetin-3-O-galactoside)	
	Isoflavons	
	Ononin [7-O- β -D-glc; 7-O-β-D -glc-(6′′-acetate)]	
	Sissotrin [(7-O- β -D-glc, 7-O- β -D-glc-(6′′-malonate)]	
	Astroside (7-O- β -D-glc)	
	Daidzin ((7-O- β -D-glc)	
	Calycosin glucosides(7-O-β-d-glucoside)	
	7-O- β -d-glucoside 6-O-malonate	
	10-dime-thoxypterocarpan-3-O- β -d-glucoside	
	7,2-dihydroxy-3,4-dimethoxyisoflavan-7-O- β -d-glucoside	
	Formononetin-7-O- β -d-*gluco*-side-6-O-malonate	
	Formononetin (7-hydroxy-4′-methoxyisoflavone)	
	Cladrin (7-hydroxy-3′,4′-dimethoxyisoflavone)	
	Afrormosin (7-hydroxy-6,4′-dimethoxyisoflavone)	
	Calycosin (7,3′-dihydroxy-4′-methoxyisoflavone)	
	Odoratin (7,3′-dihydroxy-6,4′-dimethoxyisoflavone)	
	Pratensein (5,7,3′-trihydroxy-4′-methoxyisoflavone)	
	8,3′-dihydroxy-7,4′-dimethoxyisoflavone	
	7,5′-dihydroxy-3′-methoxyisoflavone	
	7,3′-dihydroxy-8,4′-dimethoxyisoflavone	
	Isoflavanes	
	Astraisoflavanin	
	Astraganoside	
	5′-hydroxy-isomucronulatol	
	7-O-methylisomucronulatol	
	(3R)-7,2′,3′-trihydroxy −4′-methoxyisoflavane	
	(3R)-2′-hydroxy-7,3′,4′-trimethoxyisoflavane	
	(3R)-7,2′-dihydroxy-5′,6′-dimethoxyisoflavane	
	(3R)-8,2′-dihydroxy-7,4′-dimethoxyisoflavane	
	(3R,4R)-3-(2-hydroxy-3,4-dimethoxyphenyl)-chroman-4,7-diol	
Flavonoids	**Chalcones**	Li et al. (2014)
	4,2′,4′-trihydroxy-3′-prenylchalcon	Bratkov, Shkondrov,
	2′,4′-dihydroxy-3,4-dimethoxychalcon	Zdraveva, & Krasteva,
	4,2′,4′-trihydroxychalcon (isoliquiricigenin)	(2016)
	2′,4′-dihydroxy-2,3-dimethoxychalcon	Li et al. (2019a)
	2′,4′-dihydroxy-4-methoxychalcon	Li et al. (2019b)
	Pterocarpans	
	Medicarpin (3-hydroxy-9-methoxypterocarpan)	
	Methylnissolin (6aR,11aR)-3-hydroxy-9,10-dimethoxypterocarpan)	

Table 1 (*continued*)

Groups	Compounds	References
	(6aR,11aR)-10-hydroxy-3,9-dimethoxypterocarpan	
	(6aR,11aR)-3,9,10-trimethoxypterocarpan	
Saponins	**Cyclortane-type triperpenoids**	Lee et al. (2013)
	Astragaloside III	Fu et al. (2014)
	Astramembranosides A	Li et al. (2014)
	Astramembranosides B	Li et al. (2019b)
	Huangqiyegenin III	
	Huangqiyegenin IV	
	Huangqiyenin E	
	Huangqiyenin F	
	Saponin-Huangqiyiesaponin C	
	Trideacetylhuangqiyegenin III	
	Cycloartane-type triterpene glycoside	
	Agroastragaloside V	
	Astragalus saponins and Cycloartane glycosides	
	β-sitosterol	
	β-daucosterol	
	Sphondin	
	Astragalus saponin I–IV	
	Astragalus saponins VI–VIII	
	acetyl Astragalus saponin I	
	isoastragaloside IV	
	Agroastragaloside I–IV	
	Astragalus aglycone	
	Cyclocanthoside A	
	Asernestioside C	
	Calycosin-7-O-β-D-glucoside	
	6,3′-dihydroxy-2′,4′-dimethoxyisoflavan-6-O-β-D-glucopyranoside	
	7,3′-dihydroxy-6,4′-dimethoxyisoflavon-7-O-β-D-glucopyranoside	
	3-O-β-Dxylopyraosyl-24S-cycloart-3β,6α,16β,24,25-pentaol-25-O-β-D-glucopyranoside	

distinguish the pathogenic cells and normal dendritic cells, thus provide proper response to fight against it. AM compounds also protect and proliferate activity of natural killer cells (NK) to fight other disease cells. With all these mechanism pathways, the immunomodulation and protection imply its effects on diseases and harmful agents such as cancers, asthma, radiation, and harmful microorganisms (virus, parasites) (Chen et al., 2020). For instance, extract compound from AM called PG2 improve immune response to tumor via downregulation Programmed Cell Death Protein Ligand-1 (PD-L1) expression (Chang, Kuo, et al., 2020). Similar study from (Chang, Tsai, et al., 2020) found the effect of APS on inhibition of tumor by blocking the activity of f PD-L1-induced T cell exhaustion in peripheral blood mononuclear cells in mice. A recent study of AM used for asthma treatment (*Budesonide* and *Terbutaline*) also produced better effects on reducing airway inflammation and regulation of Treg/Th17 cell balance in asthmatic children (Wei, Quing-Bing, & Wei, 2019). Furthermore, AM and its bioactive compounds have been proved immunomodulation not just *in vitro*, but also in animal model studies. For instance, of the supplementation of AM powder up to 300 mg/kg diet showed the improvement in immune organs and its functions (liver and kidney) in broilers, while APS at 10 g/kg proliferated growth rate and intestinal development of young chicks (Farag & Alagawany, 2019). Other animal models such as fish, monogastric, and ruminant animals also showed positive effect of AM and the bioactive compounds on improving immune protection as well as general growth performance (Cheng, Lei, & Kim, 2020; Hao et al., 2020; Wu, 2020).

Furthermore, AM and its bioactive compounds have been proved immunomodulation not just *in vitro*, but also at animal model study. For instance, of the supplementation of AM powder up to 300 mg/kg diet showed the improvement in immune organs and its functions (liver and kidney) in broilers, while APS at 10 g/kg proliferated growth rate and intestinal development of young chicks (Farag & Alagawany, 2019).

Another important effect of this herb on improving health and curing

diseases is its anti-inflammatory property. Auyeung, Han, and Ko (2016) reviewed the anti-inflammatory effects of AM and its active compounds such as APS, flavonoids, and saponins. Moreover, another prominent active compound, AS-IV can inhibit airway inflammation associated with asthma. AS-IV exerts anti-asthmatic effects via the inhibition of mTORC1 signaling pathway of BALB/c mice by reducing the inflammatory cytokines (IL-4, IL-5, and IL-17). It also reduced inflammatory cell infiltration in the lung tissues (Jin et al., 2017). AS-IV helps protect kidney cells from inflammation in renal fibrosis by the expression of pro-inflammatory through TLR4/NF-kB signaling pathway (Zhou, Sun, et al., 2017). This compound can also suppress inflammation of the inner lining of the uterus on lipopolysaccharide (LPS)-induced endometritis via reduction of pro-inflammatory cytokines production (IL-1β and tumor necrosis factor alpha, TNF-α) and its mediators (nitric oxide, NO and myeloperoxidase, MPO) by repressing NF-kB, p38, and JNK signaling pathways (Wang, Chen, et al., 2019). APS can reduce bowel inflammation through the mechanism of attenuating NF-kB phosphorylation and reduce pro-inflammatory cytokines production (TNF-α, IL-1β, IL-6, and IL-17) and its mediator (MPO) in colonic tissues (Lv et al., 2017). Moreover, AM extract can promote intestinal cell reparation by inhibiting the expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) and enzymes responsible for cell inflammatory such as cycloxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). It also decreased the inflammatory biological marker nitrotyrosine, NF-κB action, and reactive oxygen species (ROS) in intestinal cells (Adesso, Russo, Quaroni, & Marzocco, 2018; Cui et al., 2018), and diminish the secretion of inflammatory cytokines caused by allergens (Bing et al., 2019). AM also seems to have cardio-protective properties. Astragalus polysaccharides lowered the plasma lipids in rats, which can be potential for cardio health (Pan, Gao, & Wu, 2017). The flavonoids from AM lead to vasorelaxation as well as protect cardiac and vascular endothelial cells via the Akt/eNOS signaling pathway (Liu, Zhao, & Luo, 2017). The compound AS-IV reduces cardiac muscle injury both *in vitro* and *in vivo* by reducing myocyte apoptosis throughout decreasing lactate dehydrogenase, calcium-sensing receptor expression (CaSR), and upregulation extracellular signal-regulated kinase 1/2 (ERK1/2) (Yin, Hou, & Lu, 2018). It also inhibits the cardiac muscle cells injury linked to hypoxia-reoxygenation (Yang, Zhou, Xia, Yao, & Chang, 2019). In TCM, AM is also known as an anti-aging intervention (Zhao & Luo, 2017). A study of APS on mice (D-galactose 100 mg/kg/d, 7 weeks), reduced brain mitochondrial oxidative stress, which in turn slowed the aging process (Li, Zhang, et al., 2012). A later review by Liu, Zhao, and Luo (2017) detailed the anti-aging effects of AM, related to the extracted compound, called *TA-65,* which increases telomerase activity and has age-reversal/protective effects in immune system cells. The authors also described the potential of AM compounds such as APS, flavonoids, and saponin at promoting anti-aging effects. Through oxidative stress reduction in cells, immunomodulatory effects, and various protective effects, AM can promote longer lifespans and greater organ longevity (e.g., vascular cells, brain cells, etc.). Based on the above-described health benefits there have been numerous studies that have demonstrated the potential uses of AM relative to neurodegenerative diseases, diabetes and cancer.

3.2. Neurodegenerative diseases

Alzheimer's disease (AD) and *Parkinson's disease* (PD) are the common neurodegenerative diseases. Many people, especially elderly people, suffer from these diseases. There are many factors that contribute to the development of these diseases including genetic disorders, iron imbalance, oxidative stress, defective neuron mitochondria, and inflammation of neurons (Xie, Gao, Xu, & Meng, 2014). Based on many studies, *A. membranaceus* and its bioactive compounds have been found to be potent at treating these conditions.

Astragaloside IV is a well-known AM compound, known for its neuroprotective effects. It acts as an antioxidant and reduces cell inflammation and oxidative stress. The main cause associated with PD is the deterioration of dopamine neurons in *substantia nigra* (Haavik & Toska, 1998). *Astragaloside* IV helps protecting dopaminergic neurons and promotes neural process outgrowth, which is potentially therapeutic in PD (Chan et al., 2009). A recent large review by Costa et al. (2018) described all the benefits of this compound for neurodegenerative diseases such as AD, PD, cerebral ischemia, and autoimmune encephalomyelitis. AS-IV maintains dopamine synthesis by increasing enzymes tyrosine hydroxylase (TH) and nitric oxide synthase (NOS) which are involved in neuron production pathways, neural signaling, and cell immune responses. The other mechanism of AS-IV in PD disease is the prevention of neuron pathology by a neurotoxicant called 1-methyl-4-phenylpyridium ion (MPP$^+$) throughout the increasing cell survival, inhibiting pro-apoptosis Bax-mediated pathway and caspase protein (Caspase-3) activity which can destroy cellular structures while increasing anti-apoptosis B-cell lymphoma 2 (Bcl-2). It also protects neuron mitochondria from apoptosis and DNA damage by inducing Akt to promote binding of hexokinase-II (HK-II) and mitochondrial for protection of neural mitochondria (Li, Yang, et al., 2019).

In AD, AS-IV can reduce neuro-pathogenesis caused by amyloid β-peptide (Aβ) by the diminution of intracellular reactive oxygen species (ROS), mitochondrial peroxide, and inhibit pro-apoptosis Bax expression and caspase-3. In contrast, it increases anti-apoptosis Bcl-2 and improves cell regeneration by enhancing mitochondrial membrane and ATP production. This mechanism helps reduce mitochondrial permeability, which prevents cell death via mitochondrial dysfunction (Sun et al., 2014). APS also helps reduce metabolic stress and inflammation caused by plaque in the brain (Huang et al., 2017). As described in the review by Costa et al. (2018), AS-IV plays important role in reducing neuro-pathogenesis, apoptosis, and inflammatory in AD. The AS-IV reduces Aβ plaques by increasing peroxisome proliferator-activated receptors (PPARs) which is an anti-inflammatory nuclear receptor protein and inhibits β-secretase 1 or known as beta-site amyloid precursor protein cleaving enzyme 1 (BACE1). This can prevent the destruction of mitochondrial permeability transition pore (mPTP), thus it prevents the Aβ plaques and protect neuron mitochondria. Moreover, it reduces mitochondrial dysfunction in cortical cells by inhibiting protein phosphoinositide 3-kinase (PI3K) which are associated with the production of pro-inflammatory cytokines. It also promotes neural stem cells (NSCs) and differentiation between neural Nestin and GFAP cells that is beneficial for the learning process and improves memory. The latest research on APS showed a reduction in iron overload, which helps improve cognitive functions, thus slowing the pathological development of AD by bringing balance to normal iron homeostasis (Zhang, Kung, & Chai, 2018). This study also demonstrated the decrease of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) and increase of glutathione peroxidase (GPx) and superoxide dismutase (SOD), which helps reduce oxidative stress and inflammation in cerebral cortex. Zhang et al. (2019) found that APS, at dose 20 mg/kg, has the potential to treat diabetic cognitive impairment by improving cognitive functions in rats with induced diabetes. This benefit is good for both treating neuron impairment and reducing the severity of diabetes.

3.3. Anti-diabetes

Diabetes mellitus (DM) is a globally prevalent disease that afflicts a large number of people. It is a metabolic disorder that causes chronic high blood glucose levels due to the failure of insulin activity and destruction of beta cells (Ozougwu, Obimba, Belonwu, & Unakalamba, 2013). Agyemang et al. (2013) reviewed the effects of AM and its bioactive compounds on both type 1 and 2 *diabetes mellitus*. Type 1 DM results from dysfunction of autoimmune systems that destroying pancreatic beta cells. APS play the main role in reducing effects of Type 1 DM. It protects pancreatic beta cells from autoimmune-linked cell death throughout the mechanisms of anti-oxidation, immunomodulatory, anti-inflammation, and anti-apoptosis. It helps balance type 1 and 2 helper T cells (T$_h$1 and T$_h$2), which stimulates the inflammatory

response and promotes antioxidant activity against apoptosis of pancreatic beta cells. APS reduce the expression of T_h1 cytokines (IL-12 and TNF-α, interferon gamma IFN-γ) and increase T_h2 cytokines (IL-4, IL-5, IL-6, and IL-10), thus decreased Th1/Th2 expression which lowered intracellular autoimmunity and inflammatory responses. Moreover, APS increase peroxisome proliferator-activated receptor gamma (PPAR-γ) and SOD and decrease iNOS and NO which can reduce oxidative stress (antioxidant) and acting as anti-inflammatory. It also displays the effects on death of beta cells via inhibition the enzyme caspase-3, improves Bcl-2 expression, increase galectin-1, and decrease CD^{4+} and CD^{8+}.

Type 2 DM is caused by insulin resistance and insufficient glucose metabolism (Nie, Chen, Hu, & Fan, 2019). The active compounds of AM have positive effects on regulating the mechanism of glucose and lipid metabolisms and improve the insulin function, thus exhibit hypoglycemia effects in diabetes. The most prevalent compounds, APS improve glucose transportation and reduce its accumulation in skeletal muscle and fat. The mechanism of this activity increases the expression and activity of insulin-regulated glucose transporter called Glucose Transporter Protein Type 4 (GLUT4). Moreover, APS induces several enzymes associating with glucose metabolism such as hepatic glycogen synthase (GS), adenosine monophosphate-alpha (AMP-α), and acetyl-CoA carboxylase. APS also improve fatty acid catabolism in type 2 DM via enhancing expression of PPAR-α. Flavonoids, saponins (*Astragaloside-II*, *isoAstragaloside-I*), and APS increase adiponectin, adipo-R1, and adenosine monophosphate protein kinase (AMPK) which are involved in glucose and lipid metabolism in liver, muscle, and adipocytes. APS have indirect effects on sensitizing insulin signaling by diminishing *endoplasmic reticulum* (ER) stress. It reduces the expression and activity of protein kinase that is a negative regulator of insulin receptor signal transduction known as protein kinase-like endoplasmic reticulum kinase (PERK). This mechanism is further affected by the inhibition of activating transcription factor-6 (ATF-6) by reducing expression and activity of protein tyrosine phosphatase-1-B (PTP1B). It also reduces the transcription repressor proteins such as XhoI site-binding protein 1 (XBP1) and glycogen synthase kinase 3 beta (GSK3β). APS affect insulin resistance by decreasing expression of an insulin-resistance protein, resistin. Thus, improve insulin sensitivity. It also improves insulin signal transduction by increasing receptor substrate-1 (IRS-1) and beta transmembrane receptor (IR-β) in muscle cells (Agyemang et al., 2013). Venkatakrishnan, Chiu, and Wang (2019) strongly recommended using *Astragalus* for management of Type 2 DM based on its effects on glycaemia. All major components of AM (i.e., polysaccharides, saponins, and flavonoids fractions) lower high blood glucose levels, by restoring and inducing insulin pathways to work properly, which improve the functional ability of pancreatic beta cells. Research conducted on diabetic mice found that a combination of APS with *Crataegus* flavonoids, administered orally at 200 mg/kg/day, improved pancreatic islet cell and liver cell metabolism (Cui, Zhang, Jiang, & Xie, 2016). A recently discovered new polysaccharide extracted from AM, called *AERP* (Molecular weight of 2.01×10^6 Da) has found to have hypoglycemic effects on diabetic mice. It reduced hyperglycemia by preventing tissue impairment and a decline in cognitive function (Liu, Liu, et al., 2019). You et al. (2019) recently found that *Astragaloside* IV had protective effects against high blood glucose-induced changes in human umbilical vein endothelial cells (HUVEC) injury. *Astragaloside* IV inhibits endothelial cell dysfunction in diabetics by suppressing apoptosis and inflammatory reactions throughout the body by inhibition of the *c-Jun N-terminal kinase* (JNK) signaling pathway. It reduced expression of TNFα. Similar studies showed that *Astragaloside* IV disrupts glucose-induced changes in renal tubular epithelial-mesenchymal by impeding epithelial HK-2 cells and blocking mTORC1/p70S6K signaling pathways, which causes high glucose activation (Chen, Yang, et al., 2019). *A. membranaceus* has also shown to clinically improve diabetes-related problems, particularly those associated with *Chronic Kidney Disease* (CKD). Huang, Su, Sun, and Huang (2018) reviewed 550 CKD patients

taking TCM, containing AM or AM as a single herb at a dose of 1.5 g/day, for up to an 11.9-day period. Compare to non-TCM, these patients have higher survival rates, up to 80% for a 12-year follow-up period.

3.4. Anticancer and antitumor

Cancer is a deadly disease linked to huge worldwide mortality (Siegel, Miller, & Jemal, 2020). It is caused by abnormal cell growth and can happen in almost any part of an organism, from small tissues and groups of cells (leukemia, lymphoma, myeloma) to major organs and body parts (digestive system, respiratory system, genital system, urinary system, skin, breast, brain, etc.) (Miller et al., 2019; Siegel, Miller, & Jemal, 2020). *A. membranaceus* and its extracts or selective bioactive compounds led researchers to find its potential uses for anti-carcinogenesis properties for cancer such as gastric cancer, colon cancer, lung cancer, liver cancer, breast cancer, etc. There have been numerous *in vitro* and *in vivo* studies on the anti-tumor and anti-cancer effects of AM. Cho and Leung (2007) reviewed the *in vitro* and *in vivo* anti-tumor effects of AM and its bioactive components. The authors indicated the anti-tumor mechanism of a compound called *AI* in human and murine cells. The compound was identified from combination of hydro-distillation and ethanol crude extraction, characterized by its presence at the highest peak of radioactivity (3880± 150 cpm) at concentration of 100 *μg*/ml. The mechanism of anti-tumor effects was associated with the intervention of the immune response by inhibiting tumor cell growth, preventing inflammation, and restoring tumor suppression throughout host mediation. *AI* enhances the production of TNF to play its role in killing cancer cells in tumor-bearing mice. It influences the monocytes for cell differentiation. It also triggers Lymphokine-activated killer (LAK-like) activity to toxify cancer cells. An extract of APS (fucose, arabinose, galactose, glucose, and xylose), at an oral dose of 300 mg/kg, in mice, suppressed the tumor growth rate by almost 50%. It also protects immune organs, ameliorates macrophage activity, and causes the proliferation of Natural Killer and T cells mice with tumors (Liu, Yu, et al., 2018).

Many anti-tumor effects of this herb on the digestive system have also been discovered. For instance, Wang et al. (2013) found the apoptogenic property of *Astragalus* saponins (AST) acted on human gastric carcinoma cell growth (BGC-823) *in vitro* and *in vivo* via inhibition of cancer cell invasion in G0/G1 phase of cell cycle. Other compounds, such as APS and flavonoids, also have distinctive anti-cancer effects. APS promotes immune-restorative and immunomodulating activity, while *Astragalus* flavonoids are mainly responsible for the antioxidant and anti-inflammatory effects, which are protective and anti-inflammation agents, respectively, that can help prevent gastrointestinal cancer (Auyeung, Han, & Ko, 2016). A very recent study showed the effect of APS4, extracted from cold water combined with cryo-concentration method and ethanol precipitation on apoptosis of human gastric carcinoma cells (MGC-803) via intrinsic mitochondrial pathways (Yu, Ji, Dong, Feng, & Liu, 2019). The mechanism of apoptosis explained via deterioration of mitochondrial of cancer cell after the APS4 captured the MGC-803 cell in S shape cell cycle and cause the cell death by accumulating ROS, increase Bax/Bcl-2 ratio, activating caspase-9/-3 by cytochrome *c* releasing, and breaking down poly-ADP-ribose polymerase (PARP) of this cancer cell.

Anti-cancer properties of AM have also been found in other important organs. Huang and his group found that APS affected apoptosis of human hepatocellular carcinoma (HCC) cells via suppression activity of Notch Receptor 1. The mechanism lies between decreasing survival and enhancing apoptosis on HCC cells throughout increasing Bax/Bcl-2 expression and caspase-3/-8 (Huang, Liao, & Sun, 2016). It has also been shown to aid balancing cytokines and block immune suppressive effects of regulatory T cells throughout blockage of growth and expression and its migration in the tumor microenvironment of HCC. APS reduced T_{reg} cells CD^{4+} and CD^{25+}, resulting in balancing cytokines, particularly by suppressing diabetes-related problems stromal cell-derived factor 1 (SDF-1) and diminution the expression of Forkhead

box P3 (Foxp3) in local HCC (Qiang, Bao, Li, Zhang, & She, 2012).

AM has also been shown to have great activity against hepatic cancer when combined with other herbs. For instance, a combination of APS and curcumin has more effect on the liver cancer cell (HepG2) than using a single herb. It was found to limit tumor cell invasiveness, thus normalizing tumor vascular (Tang et al., 2019). Another recent study by Wu et al. (2019) found that the extracted bioactive compounds of AM (*Astragalosides* and APS), when combined with salvianolic acids from *Salvia miltiorrhiza*, showed inhibition against hepatocellular carcinoma HepG2 cells and TGF-β1-stimulated HepG2 cells, by increasing miR-145 to promote Smad3 phosphorylation and decreasing miR-21 expression. Zhou, Meng, and Ni (2017) found that APS significantly promoted the anti-proliferative and apoptotic effects of cisplatin on nasopharyngeal carcinoma cells by decreasing pro-apoptotic Bcl-2 and elevating anti-apoptotic (Bax and caspase-3/-9) in xenograft model. AM extracts, especially by ethyl acetate fraction were also found to have apoptogenic effects on non-small cell lung cancer (NSCLC) cells (Park & Park 2018). The mechanism of apoptosis displayed by improving expression of caspase-8/-9 and accumulation of PARP. Jia, Lv, Zhang, Wang, and Zhou (2019) researched on selected compound of AM, i.e., *Astragaloside* IV, and found that they inhibited AKT/GSK-3b/b-catenin activity, which also suppressed growth and migration of NSCLC cells. Moreover, AS-IV increased cell death as expressed by the increase of Bax and caspase-3 and the decrease of Bcl-2.

The common cancers in the female population, breast cancer and ovarian cancer have also been testing the effects of AM, and many studies found positive implications. Zhou et al. (2018) found that water extracted AM repressed cell growth of breast cancer cells (i.e., MCF-7, SK-BR-3 and MDA-MB-23) and had apoptogenic effects via the PI3K/AKT/mTOR pathway. Certain polysaccharides found in AM (Pyran-type APS, 89.75% total carbohydrate and 9.3% uronic acid) were found to inhibit the invasiveness of cancer cells (MCF-7) via activated macrophages. It mediated the effects of apoptosis via increasing NO, TNF-α, and Bax/Bcl-2 expression in order to suppress cell growth (Li, Song,

et al., 2019). Liu, Zhuang, et al. (2019) found that APS also inhibited triple-negative breast cancer cell (TBCN) invasion and improved apoptosis via inhibition of PIK3CG/AKT/BCL2 pathway. Other active compounds such as *Astragaloside* III and *Astragaloside* IV were found to help prevent breast cancer via its apoptosis property (Wang, Tang, & Chen, 2015). The AM compound, formononetin, has also found to have apoptosis properties that could inhibit the spread and invasion of ovarian cancer cells (Zhang et al., 2018).

AM has also been proved to improve many disease treatments related to chemotherapy at clinical levels. For instance, *Astragaloside* IV helps stimulate *Taxol* chemosensitivity against breast cancer cells MCF-7 and MDA-MB-231 (Zheng et al., 2018). Chen, Bi, et al. (2019) recommended the use of products containing AM integrated with chemotherapy due to its inhibition of cancer and its action as an immunosuppressant during chemotherapy. McCulloch et al. (2006) reviewed clinical *meta*-analysis (34 randomized studies, 2815 patients) on *Astragalus*-based Chinese herbal medicine combined with Advanced NSCLC platinum-based chemotherapy. This combination minimizes the risk of death, improves tumor response, and general performance; it also reduces chemotherapy toxicity. Wang et al. (2016) found similarities (17 randomized studies, 1552 patients), in a combination of an *Astragalus*-based product and platinum-based chemotherapy, in the treatment of NSCLC. The authors concluded that there was an increase in the one-, two-, and three-year survival rates and an overall improvement in the response rate to this combination.

All biological mechanisms of the main bioactive compounds in the described diseases are presented in Fig. 2.

4. *Astragalus membranaceus* potential for food application

4.1. Doses and application

When it comes to the use of herbs in food, there are many questions among food organizations and food industries regarding the potential
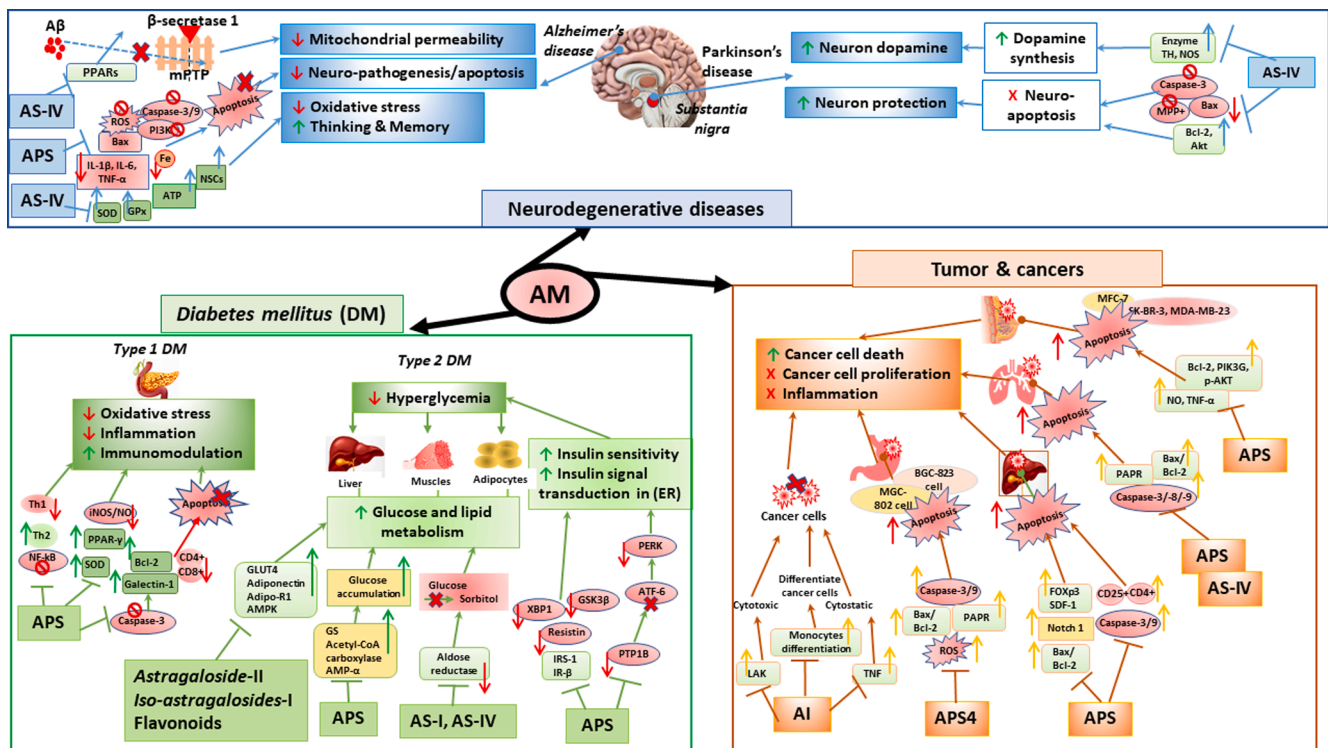

Fig. 2. Schematic presentation of the biological mechanisms of main bioactive compounds in AM (*Astragaloside-I, II, -IV, Iso-astragaloside-I, Flavonoids, Astragalus Polysaccharides, Astragalus Polysaccharides 4, AI*) on Neurodegenerative diseases (*Alzheimer's disease* and *Parkinson's disease*); *Diabetes mellitus* (Type 1 and 2); Tumors and cancers (gastric, liver, lung, and breast cancer).

Table 2

Form, doses, and methods of administrations of *Astragalus membranaceus.*

Form/function	Doses and administrations	References
Root, *Radix Astragali* as TCM	Oral: 9–30 g per day	WHO (1999)
Capsules, tablets, tea, tincture, dried root, fluid extract as TCM	Oral: 250–500 mg in capsules/tablets, 4 times per day	Cassileth and Lucarelli (2003)
Dried root as soup in remedy food	Oral: 1000 to 2000 mg/day	Micozzi and Dog (2005)
Dried root decoction	Oral: 10–30 g/day	Bone and Mills (2013)
Liquid extract or equivalent doses of the dried extract in tablet or capsule as Traditional therapeutics	Oral: 4–8 ml/day of 1:2 liquid extract	
Dried root extract, *Astragali Radix* as TCM	Oral: 0.57 g/kg body weight per day	Fu et al. (2014)
Dried root as TCM	Oral: 9–30 g dried root per day	Health Canada (2019)
Dried powder, decoction as TCM	Oral: 2–4.8 g dried root per day. Preparation as soup of dried root as remedy drink: let sit in water for 60 min and boil 20–25 min, remove and drink or dried root boil 10–15 min, remove and drink	Health Canada (2019)

role of medicinal herbs, safety, and food utilization (Chau & Wu, 2006). However, many researchers recommended the use of whole herbs in food. Tapsell et al. (2006) recommended healthy herbs and spices should not be only used for flavoring, but to fully replace or be mixed with other basic ingredients such as salt, sugar and fat. A very recent review by Opara (2019) also has shed light on the importance of including herbs as part of one's food consumption rather than just in studies of their bioactive compounds on chronic non-communicable diseases.

With all the health benefits presented and suggested by researchers, there is a strong case for including AM in the daily diet. McKenna, Hughes, and Jones (2002) reviewed that the official daily dose recommended in China (i.e., 9–30 g), which is recognized as safe when used appropriately (i.e., absent acute infection) (Bone & Mills, 2013). Moreover, according to the *State Food and Drug Administration* (FDA)*,* People's Republic of China, AM can also be used as a functional food (Chau & Wu, 2006).

The recent patent of a product called *AstraGin™*, which contains both AM and *Panax notoginseng* extracts, has been approved for a novel food application by the *European Commission* (European Commission, 2020). The suggested dose of oral intake for adults is 50 mg/day as a food supplement, which is also *Generally Recognized As Safe* (GRAS), according to the FDA. The most common forms of AM, when used as a remedy, include capsules, alcohol tincture, tablets, powder, granules, dried roots etc. In traditional Chinese medicine, the AM can be also applied as mixture with honey, see Liao et al. (2018). It is also used mainly as supplements, but not in food in Western countries (Micozzi & Dog, 2005). However, AM is also used in Asian food for daily consumption. Some other specific dose recommendations of AM, for use without an acute side effects indication are listed in Table 2.

4.2. AM reduces the side effects of disease treatments associated with food intake

A. membranaceus has not just been shown to improve health and prevent diseases; it also helps diminishing the adverse side effects of specific therapies used for disease treatment, particularly related to food intake problems. Patients with cancer, organ failure, neurodegenerative, and diabetes often also have trouble with food intake and malnutrition. For instance, in cancer patients, food intake problem is often associated with both the disease itself and the side effects of chemotherapy (Ravasco, 2005; Tseng et al., 2016). Reduced food intake and changes in food taste and flavor are common problems in cancer patients (Boltong, Keast, & Aranda, 2012). Moreover, chemotherapy causes effects such as loss of appetite, vomiting, nausea, and diarrhea, which often combine to reduce food intake and cause other general health problems (Wang et al., 2016). Studies of these problems show that AM is a notable herb in reducing the severity and maybe helping in the treatment itself. For instance, in a systematic review paper of Wang et al. (2016) on NSCLC

patients with treatment of the combination of an *Astragalus*-based product TCM (capsule or injection formulae) with platinum-based chemotherapy (17 randomized studies, 1552 patients), found reduction in side effects such as loss of appetite, nausea, weakness, anemia, neutropenia, and thrombocytopenia. Wu, Munro, Guanjian, and Liu (2005) reviewed the effect of decoctions of *Huang qi* during colorectal chemotherapy. They concluded that in colorectal cancer patients, there was a decrease in side effects such as nausea and vomiting, and lower rates of leucopenia. Lin et al. (2019) recently conducted a large *meta*-analysis (22 randomized control studies, 1409 subjects) on the combination of AM as part of TCM formulated products (oral administration as capsules, tablets, or decoction, and in injection form) or colorectal chemotherapy alone. The authors found benefits in the combination including the increase tumor response, improve general health, and especially a reduction common chemotherapy side effects such as nausea and vomiting, diarrhea, neutropenia, anemia, thrombocytopenia, and neurotoxicity. Based on these benefits alone, i.e., disease exacerbations and treatment of side effects, including AM in the diet of these patients, would be advantageous.

4.3. AM improves food quality

Improving food quality is very challenging, especially for those who have eating problems or are undergoing various disease treatments. For instance, Alzheimer's patients often have problems with taste and the loss of smell, which leads to reduced food intake and a general weakening of health (Aliani et al., 2013). Moreover, the elderlies who have chronic diseases, especially neurodegenerative diseases, often dislike foods with a strong taste, other than sweet and salty (Sergi, Bano, Pizzato, Veronese, & Manzato, 2017). This is another important reason to incorporate AM into food since it has acceptable organoleptic qualities and can sometimes even improve general food quality.

Unlike most herbs which have intense colors and strong flavors and aroma, which can mask or change the overall palatability of food when used in large amounts, AM root has a pleasant gentle scent, mild flavor, very light yellowish color, and a slightly sweet taste (WHO, 1999; Augustin & Sanguansri, 2015). This favorable property is also useful when combining AM with other ingredients. For instance, Strohalm, Houška, Novotná, and Boesenberg (2018) formulated a recipe for making hydrocolloids of 5% pulverized AM root combined with tuber fleece flower (*Polygonum multiflorum*) and red sage (*Salvia miltiorrhiza*). This recipe was accepted as a healthy food with acceptable sensorial and technological qualities (and is protected by the utility model number 31,446 in the Czech Republic).

Moreover, adding AM root powder to food can increase volume, improve texture, reduce water content, and increase the shelf life of food. Meledina, Amirova, Golovinskaia, and Ivanova (2019) formulated a wheat bread recipe containing 13–15% by weight of AM combined with other Chinese herbs. This formula improved the quality of the

bread and also reduced the staling process and made it more microbi-ologically stable, which prolonged shelf life. AM also has a slight sweetness, which is an advantage for the elderly who have problems with gustation (Sergi, Bano, Pizzato, Veronese, & Manzato, 2017).

Texture improvement is also very important for overall consumer satisfaction as well as those who have eating problems. For example, in cuisine puree, which is a common diet for those with dysphagia and those who suffer from the effects of neurodegenerative diseases, food texture is a significant issue in depressing food intake (Germain, Dufresne, & Gray-Donald, 2006; Ettinger, 2012). This can be improved by mixing AM root powder with commonly cooked and pureed vege-tables such as carrots, potatoes, pumpkins, sweet potatoes, squashes, etc. (Hotaling, 1992; Ilhamto, Keller, & Duizer, 2014). AM is a good solution for treating these problems since the formulated ready to eat food have more appetite appeal, and they are healthier, in that they contain several bioactive compounds with many health benefits.

5. Conclusions

Astragalus membranaceus has been demonstrated to have many health benefits related to wellbeing as well as preventing and treating a variety of diseases. Multiple studies support its beneficial effects on neurodegenerative diseases, diabetes, cancer, as well as general health improvement; therefore, AM should be thought of as a potentially important ingredient that can easily be incorporated into food. In addition, the advantages of its palatability characteristics and organo-leptic suitability make it uniquely applicable for improving the physi-ological characteristics of food as well as food quality in general. This can be a promising solution for simultaneously treating specific health problems and increasing food consumption in people who are receiving treatment for health problems. Further studies on formulations best suited for food and its effectiveness in patients, relative to specific dis-ease treatments, should be carried out.

6. Ethics statements**a

Our research did not include any human subjects and animal experiments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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