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Dietary *Artemisia vulgaris* meal improved growth performance, gut microbes, and immunity of growing Rex rabbits

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Abstract: *Artemisia vulgaris* (*A. vulgaris*) is an edible plant showing antioxidant and antibacterial effects, but its effect as a feed additive or forage source on the herbivore growth and health is unclear. This study aimed to investigate the effect of *A. vulgaris* meal supplementation on the growth performance, gut microbes, and immune function in rabbits. A total of 120 growing Rex rabbits were randomly allocated into 4 treatments with 6 replicates per 5 rabbits each. There were four experimental diets containing *A. vulgaris* meal at doses of 0, 3.0, 6.0 or 9.0%, respectively. The experiment lasted for 70 days. The results showed that diets supplemented with *A. vulgaris* meal improved the rabbits' feed intake, body weight gain, and decreased feed conversion ratio ($P < 0.05$). Linear and quadratic responses were found between the growth parameters and the herbal meal doses ($P \leq 0.002$). *A. vulgaris* meal also improved gut microbe populations by increasing *Lactobacilli* and *Bifidobacteria*, and decreasing *E. coli*, *C. perfringens*, *Salmonella*, and Gram-negative bacteria ($P < 0.05$), and linear and quadratic dose-dependent advantages were exhibited for these microbes ($P \leq 0.013$). Furthermore, blood levels of IgA, IgM, and lymphocytes of bursale, thymus, CD4 and CD8 were increased by the treatments containing *A. vulgaris* meal ($P < 0.05$), and linear dose-dependent effect was found on these immune indexes ($P < 0.001$). Diet supplemented with *A. vulgaris* meal is effective in improving growth, gut microbes, and immunity of Rex rabbits.

Keywords: *Bifidobacteria*; immunoglobulins; *Lactobacilli*; lymphocytes; opportunistic pathogens

Artemisia vulgaris (*A. vulgaris*) is an edible and medicinal herb in the aspects of anti-inflammation, immunomodulation, and hepatoprotection, through its secondary metabolites, mainly including flavonoids, terpenes, and phenolic acids (Abiri et al. 2018). *In vitro* studies demonstrated that *A. vulgaris* extract had good antioxidant, antibacterial, and high radical scavenging activity (Melguizo-Melguizo et al. 2014; Pandey et al. 2017). Similarly, studies in rodent showed that *A. vulgaris* had hypolipidemic, anti-inflammatory, and antioxidant properties, and oral pretreatment with this herb significantly attenuated CCl_4-induced liver damage (El-Tantawy 2015; Correa-Ferreira et al. 2017). Furthermore, *A. vulgaris* exhibited a mild antibacterial activity against *Proteus vulgaris*, *Enterococcus faecalis*, *Serratia marcescens*, and *Staphylococcus aureus*, and a non-toxic effect to-

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wards mammalian cells (Oyedemi and Coopoosamy 2015), and against endo- and ectoparasites in organic animal production (Lans and Turner 2011). Additionally, *A. vulgaris* exhibited antispasmodic and bronchodilator activities mediated through dual blockade of muscarinic receptors and calcium influx in a rabbit model (Khan and Gilani 2009).

Given the beneficial effects of *A. vulgaris* plant, coupled by its worldwide distribution and easy availability, the hypothesis of the present study is that *A. vulgaris* meal can promote growth by modulating gut bacteria and immunity in farm animals, especially herbivores, but little is known about this. The present study aimed to investigate the effect of supplemental *A. vulgaris* stem and leaf meal on the growth performance, gut opportunistic bacteria, and serum immunoglobulins of growing Rex rabbits.

MATERIAL AND METHODS

A. vulgaris meal. *A. vulgaris* plants at vegetative period from the Funiu Mountains in Songxian of China (112°10’ N, 34°15’ E) were cut above the ground, air dried, ground (20-mesh sieve) into meal (for chemical compositions see Table 1), and added at 0, 3.0, 6.0 or 9.0% in diets. The doses were selected according to the authors’ pilot study. A chemical analysis of proximate nutrients and minerals in *A. vulgaris* was carried out according to the method by Zhang (2016). Total flavonoids in *A. vulgaris* were determined by China National Food Safety (GB/T 20574-2006) and total phenolic acids were measured using a Folin–Ciocalteu assay according to Grzegorczyk-Karolak et al. (2015).

Diets. The nutrition levels of experimental diets and animal management were as recommended by the Technical Specification for Feeding and Management of Rex Rabbits (NY/T2765-2015, Ministry of Agriculture of China, 2015) (Table 2). All diets contained similar levels of nitrogen, energy, and fibre, fed as pellets (cold formed; diameter × length, 3.5 × 8.0 mm), and water content was maintained under 13%. The diets were stored in a cool, dry, dark, and well-ventilated place. No antibiotics were used either in feed or water throughout the experiment.

Table 1. Chemical composition of *A. vulgaris* meal (%)

Composition	Contents
Dry matter	87.31
Crude protein	9.26
Crude fibre	11.92
Crude fat	2.21
Crude ash	8.33
Ca	0.19
P	0.34
Flavonoids (mg/g)	13.58
Phenolic acids (mg/g)	4.25

Table 2. Ingredients and nutrient levels of diets (air-dry basis)

Items	T1	T2	T3	T4
Ingredients (%)				
Artemisia vulgaris meal	0.0	3.0	6.0	9.0
Corn	26.0	25.0	25.0	25.0
Soybean meal	12.5	13.0	13.5	13.8
Brewers dried grain	14.0	15.0	15.0	15.0
Alfalfa meal	33.5	30.0	26.5	23.0
Wheat bran	10.0	10.0	10.0	10.0
Dicalcium phosphate	2.0	2.0	2.0	2.0
Limestone	0.0	0.0	0.0	0.2
Premix[1]	2.0	2.0	2.0	2.0
Nutrients[2] (%)				
Crude protein	17.18	17.36	17.39	17.32
Digestible energy (MJ/kg)	10.81	10.80	10.81	10.81
Crude fibre	13.69	13.77	13.73	13.68
Lysine	0.75	0.77	0.78	0.78
Methionine + cysteine	0.52	0.53	0.54	0.54
Ca	1.08	1.04	1.00	1.03
P	0.59	0.59	0.60	0.60

[1]premix provided the following per kg of diets: vitamin A 12 000 IU, vitamin D 2000 IU, vitamin E 30 IU, Cu 12 mg, Fe 64 mg, Mn 56 mg, Zn 60 mg, I 1.2 mg, Se 0.4 mg, Co 0.4 mg, NaCl 6.4 g

[2]calculated by the Chinese Feed Database, version 21, 2010

Animal management. The experimental protocol of this study was approved by the Institutional Committee for Animal Use and Ethics of Henan University of Science and Technology. One hundred twenty weaned male Rex rabbits at approximately 35 days of age with initial body weight (BW) 750 ± 3.7 g (mean ± SD) were randomly assigned to four dietary treatments. There were 6 replicates in a treatment and 5 rabbits per replicate. All rabbits were housed individually in cages (length × width × height, 35 × 45 × 40 cm^3)

https://doi.org/10.17221/162/2018-CJAS

and had free access to diets and water. The feeding trial lasted for 70 days. Rabbits and feeds in each replicate were weighed at 35, 70 and 105 days of age. Average feed intake (FI), average body weight gain (BWG), and feed conversion ratio (FCR) were immediately adjusted when mortality occurred. All the rabbits were monitored for general health at least twice a day.

Sample collection. At the end of the trial, rabbits per replicate were weighed and blood was taken from the ear vein of each rabbit into two tubes. One tube of blood was then centrifuged at 3000 *g* for 15 min to obtain serum for the analysis of immunoglobulins, and another heparinized evacuated tube was used to obtain whole blood for T and B lymphocyte tests. Fresh feces from recta without contamination were collected and stored at –40°C for gut microbe enumeration.

Lymphocyte detection. Percentages of T and B lymphocytes in the whole blood were quantified using the method of E-rosette formation tests described by Brain et al. (1970). Blood mononuclear cells were isolated as described above and adjusted to a concentration of 1×10^7/ml with calcium and magnesium-free phosphate buffer saline buffer (pH 7.4) for determining the percentages of CD4 and CD8 T lymphocytes according to the method by Liu et al. (2008). Concentrations of IgA, IgG, and IgM in serum were measured using commercial kits for IgA (H108), IgM (E025), and IgG (E026) from the Nanjing Jiancheng Biological Institute (Nanjing, China).

Bacterial enumeration. Each rectal content (1 g) was diluted with sterile buffered peptone water (0.1%, 9 ml, 0–4°C) and mixed. The suspension of each sample was serially diluted between 10^{-1} to 10^{-7} dilutions, and each diluted sample (100 μl) was subsequently spread onto duplicate selective agar plates for bacterial counting. The number of colony-forming units (CFU) was expressed as a logarithmic ($\log_{10}$) transformation per gram of intestinal digesta. Fecal bacterial populations were detected using commercial media including *Lactobacillus* selective agar (HB0392), *Bifidobacterium* selective medium (HB0934), *Escherichia coli* (*E. coli*) chromogenic medium (HB7001), *Clostridium perfringens* (*C. perfringens*) sulfite polymixin sulphadiazine agar base (HB0256), *Salmonella* deoxycholate hydrogen sulfide lactose agar (HB4087), and Gram-negative bacteria (Gram$^-$) selection medium (HB8643). The media were purchased from Qingdao Hope Bio-Technology Co., Ltd. (Shandong, China).

Statistical analysis. Data were analyzed using contrasts of one-way ANOVA procedure (IBM SPSS, Armonk, USA). Linear and quadratic equations of polynomial contrasts were used for the analysis of dose trends of *A. vulgaris* meal at 0, 3.0, 6.0, and 9.0%. The average of 5 rabbits per replicate was the statistical unit for growth performance, gut bacteria ($\log_{10}$ CFU), and blood immune parameters. Differences of variables were separated using Tukey's *b* test at $P < 0.05$ level of significance.

RESULTS AND DISCUSSION

Diets supplemented with *A. vulgaris* meal improved final BW, FI, and BWG, and decreased FCR of 35–105-day old Rex rabbits ($P < 0.05$) (Table 3). As known, *A. vulgaris* is one of important medicinal plants rich in volatile oils, and has a long history in treating human ailments in many countries

Table 3. Effect of *Artemisia vulgaris* meal on the growth performance of Rex rabbits

Item	*Artemisia vulgaris* meal (%)				SEM	*P*-value	
	0.0	3.0	6.0	9.0		linear	quadratic
Initial BW (g/rabbit)	749.8	750.0	750.3	748.7	0.76		
Final BW (g/rabbit)	2244^b	2341^a	2352^a	2349^a	9.85	< 0.001	< 0.001
FI (g/rabbit)	6055^c	6262^b	6302^a	6302^a	21.60	< 0.001	< 0.001
BWG (g/rabbit)	1494^b	1591^a	1602^a	1601^a	9.86	< 0.001	< 0.001
FCR	4.052^a	3.938^b	3.935^b	3.938^b	0.01	< 0.001	0.002

BW = body weight, FI = feed intake, BWG = body weight gain, FCR = feed conversion ratio (FI/BWG), SEM = standard error of the means

$^{a–c}$means within a row not sharing a superscript were significantly different ($P < 0.05$)

https://doi.org/10.17221/162/2018-CJAS

Table 4. Effect of *Artemisia vulgaris* meal on the gut bacteria of Rex rabbits

Item	*Artemisia vulgaris* meal (%)				SEM	*P*-value	
	0	3.0	6.0	9.0		linear	quadratic
Beneficial bacteria ($\log_{10}$ CFU/g of feces)							
Lactobacilli	6.64[b]	6.95[a]	7.18[a]	7.12[a]	0.054	< 0.001	0.013
Bifidobacteria	4.79[b]	5.79[a]	5.78[a]	6.00[a]	0.115	< 0.001	0.007
Opportunistic bacteria ($\log_{10}$ CFU/g of feces)							
E. coli	4.67[a]	4.30[b]	3.88[c]	3.97[c]	0.071	< 0.001	0.001
C. perfringens	1.76[a]	1.02[b]	0.93[bc]	0.79[c]	0.083	< 0.001	< 0.001
Salmonella	1.30[a]	1.12[b]	1.07[b]	1.09[b]	0.022	< 0.001	< 0.001
Gram$^-$	6.88[a]	6.14[b]	5.97[b]	5.64[c]	0.099	< 0.001	0.002

SEM = standard error of the means, CFU = colony-forming units

[a–c]means within a row not sharing a superscript were significantly different ($P < 0.05$)

(Zhigzhitzhapova et al. 2016; Correa-Ferreira et al. 2017; Abiri et al. 2018). Considering its wide distribution, high yields, and effective medicinal values, *A. vulgaris* meal can be an alternative to antibiotics in improving animal growth. Indeed, this was first demonstrated in the present study. Furthermore, there were linear and quadratic responses of final BW, FI, BWG, and FCR to *A. vulgaris* meal doses ($P \leq 0.002$), and the doses of 6.0 and 9.0% showed better effect on FI than the dose of 3.0% ($P < 0.05$). Additionally, in the present study, no mortality and typical symptoms of diseases occurred throughout the experiment, but the improved growth by *A. vulgaris* supplementation also indicated its growth-promoting potential.

Compared with the control treatment, *A. vulgaris* meal added at 3.0, 6.0 or 9.0% increased the gut beneficial populations, *Lactobacilli* and *Bifidobacteria*, of Rex rabbits ($P < 0.05$), and decreased opportunistic pathogenic bacteria, including *E. coli*, *C. perfringens*, *Salmonella*, and Gram$^-$ ($P < 0.05$) (Table 4). Furthermore, the beneficial regulation effects on these bacteria responded linearly and quadratically to the increasing doses of *A. vulgaris* ($P \leq 0.013$). Literature has shown that hydroalcoholic extract from the whole parts of *A. vulgaris* exhibited a mild antibacterial activity against *P. vulgaris* ATCC 6830, *E. faecalis* ATCC 29212, *S. mercescens* ATCC 9986, *S. aureus* OK1 and OK3, and exhibited non-toxic effect towards mammalian cells, which in part supported its medicinal uses in folklore medicinal system (Oyedemi and Coopoosamy 2015).

Additionally, the *A. vulgaris* oil also exhibited strong antimicrobial activity against plant pathogens and insecticidal activity against insect pests (Badea and Delian 2014). In farm animals, however, experimental studies on the antibacterial effect of *A. vulgaris* are unavailable. Major components in *A. vulgaris* oil are 1,8-cineole, beta-pinene, thujone, artemisia ketone, camphor, caryophyllene, camphene, and germacrene D, and the increasing interest in using the oil as an antimicrobial agent in humans is mainly due to its natural origin, wide spectrum of activity and its generally recognized safe status (reviewed by Pandey and Singh 2017). The present study first reported that diets supplemented with whole *A. vulgaris* plant meal beneficially regulated gut microbiota of Rex rabbits, but its antibacterial potential on skin or wools warrants further study.

The serum levels of IgA and IgM were increased by the addition of *A. vulgaris*, and the effects by 6.0 and 9.0% doses were more pronounced than by the 3.0% dose ($P < 0.05$) (Table 5). Furthermore, with the increasing doses of *A. vulgaris*, IgA and IgM exhibited linearly and quadratically increasing trends ($P < 0.001$). These findings indicated the enhancing function of *A. vulgaris* on the innate immunity of Rex rabbits. As currently known, *A. vulgaris* is a rich source of monoterpene, sesquiterpene, phenolic and flavonoids compounds, and those phytochemicals contribute to a good antioxidant and antibacterial activity (Karabegovic et al. 2011; Melguizo-Melguizo et al. 2014; Pandey et al. 2017). Also, methanolic leaf extract of *A. vulgaris* at doses of 200 or 400 mg/kg BW after surgical insertion of cotton pellets into groin region of rats showed a significant anti-inflammatory function (Afsar et al. 2013). To date, the effect of *A. vulgaris* on the immunity of rats or other animals has been unclear. The present study results indicate

https://doi.org/10.17221/162/2018-CJAS

Table 5. Effect of *Artemisia vulgaris* meal on the blood immunoglobulins and lymphocytes of Rex rabbits

Item	*Artemisia vulgaris* meal (%)				SEM	*P*-value	
	0	3.0	6.0	9.0		linear	quadratic
Serum immunoglobulins (g/l)							
IgA	0.26[c]	0.35[b]	0.38[a]	0.39[a]	0.012	< 0.001	< 0.001
IgM	0.20[c]	0.28[b]	0.31[a]	0.31[a]	0.010	< 0.001	< 0.001
IgG	0.20	0.21	0.22	0.22	0.004	0.017	0.448
Whole blood lymphocytes and subsets (%)							
B lymphocytes	13.85[d]	19.01[c]	20.76[b]	22.23[a]	0.684	< 0.001	< 0.001
T lymphocytes	30.15[c]	35.44[b]	35.77[b]	39.16[a]	0.709	< 0.001	0.064
CD4	21.80[c]	26.97[b]	27.62[b]	30.58[a]	0.699	< 0.001	0.038
CD8	12.76[b]	15.75[a]	15.96[a]	16.52[a]	0.345	< 0.001	0.002
CD4/CD8	1.68	1.70	1.81	1.83	0.030	0.054	0.995

SEM = standard error of the means

[a–d]means within a row not sharing a superscript were significantly different ($P < 0.05$)

that the antioxidant and anti-inflammatory property of *A. vulgaris* further contributes to the immune function of rabbits.

The percentages of B and T lymphocytes also increased by the addition of *A. vulgaris* ($P < 0.05$) (Table 5). With the increment of *A. vulgaris* doses from 0 to 9.0%, its effect on B cells significantly increased ($P < 0.05$), and in the meantime, linear and quadratic effects were found between the dependent and independent variables ($P < 0.001$). The B lymphocytes are the base of humoral immunity, so in the present study the increased percentage of B lymphocytes in the *A. vulgaris* treatments indicated the immunopotentiation of the supplement. Likewise, there have been no reports about the effect of *A. vulgaris* on the lymphocyte percentages and related immunity. El-Tantawy (2015) found that *A. vulgaris* affected the metabolism of rats on a high fat diet (100 mg/kg per day) resulting in normalized serum lipid profile, a significant increase in paraoxonase-1 activity, and decreases in serum malondialdehyde, nitric oxide, tumor necrosis factor-alpha level, and hydroxymethylglutaryl-CoA reductase activity. In the future, it will be interesting to investigate how the specific bioactive components of *A. vulgaris* regulate the cellular immunity and metabolism.

Furthermore, CD4 and CD8, the subsets of T lymphocytes, were increased by the addition of *A. vulgaris* ($P < 0.05$), and the effect of the 9.0% dose was more pronounced than that of the 3.0 and 6.0% doses for T and CD4 lymphocytes ($P < 0.05$), but the CD4/CD8 ratio was not affected by the addition of *A. vulgaris* meal. A linear effect was found on T lymphocytes ($P < 0.001$), and linear and quadratic effects were found on CD4 and CD8 ($P \leq 0.038$). CD4 lymphocytes help coordinate the immune response by stimulating other immune cells, such as macrophages, B lymphocytes, and CD8, to fight infection, and CD8 is a cell surface glycoprotein and a member of immunoglobulin supergene family that is involved in the mediation of cell–cell interactions within the immune system (Koretzky 2010). The literature about the effect of *A. vulgaris* on CD4 and CD8 lymphocytes is unavailable, but it can be deduced from the reports on similar phytochemicals, such as flavonoids and phenolic. It was reported that flavonoids modulated Th1/Th2 cytokine balance and CD4/CD8 lymphocytes ratio and decreased inflammatory mediator expressions levels in animal models (reviewed by Gandhi et al. 2018). Additionally, herbal phenolic administration increased interleukin-2 levels, tumor necrosis factor-α production, CD4/CD8 ratio, natural killer cells levels, superoxide dismutase and glutathione peroxidase activities, and decreased malondialdehyde content (Sun et al. 2017).

CONCLUSION

The diets supplemented with *A. vulgaris* meal at 3.0, 6.0 or 9.0% improved the growth performance, gut beneficial microbiota, and humoral and cellular immunity, and decreased gut opportunistic bacteria of growing Rex rabbits. The results suggest that *A. vulgaris* can serve as a natural growth-promoting additive in farm animals.

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REFERENCES

Abiri R., Silva A.L.M., de Mesquita L.S.S., de Mesquita J.W.C., Atabaki N., de Almeida Jr. E.B., Shaharuddin N.A., Malik S. (2018): Towards a better understanding of Artemisia vulgaris: Botany, phytochemistry, pharmacological and biotechnological potential. Food Research International, 109, 403–415.

Afsar S.K., Kumar K.R., Gopal J.V., Raveesha P. (2013): Assessment of anti-inflammatory activity of Artemisia vulgaris leaves by cotton pellet granuloma method in Wistar albino rats. Journal of Pharmacy Research, 7, 463–467.

Badea M.L., Delian E. (2014): In vitro antifungal activity of the essential oils from Artemisia spp. L. on Sclerotinia sclerotiorum. Romanian Biotechnology Letter, 19, 9345–9352.

Brain P., Gordon J., Willets W.A. (1970): Rosette formation by peripheral lymphocytes. Clinical Experimental Immunology, 6, 681–688.

Correa-Ferreira M.L., Verdan M.H., Livero F.A.D., Galuppo L.F., Telles J.E.Q., Stefanello M.E.A., Acco A., Petkowicz C.L.D. (2017): Inulin-type fructan and infusion of Artemisia vulgaris protect the liver against carbon tetrachloride-induced liver injury. Phytomedicine, 24, 68–76.

El-Tantawy W.H. (2015): Biochemical effects, hypolipidemic and anti-inflammatory activities of Artemisia vulgaris extract in hypercholesterolemic rats. Journal of Clinical Biochemistry and Nutrition, 57, 33–38.

Gandhi G.R., Neta M.T.S.L, Sathiyabama R.G., Quintans J.S.S., de Oliveira e Silva A.M., Araujo A.A.S., Narain N., Junior L.J.Q., Gurgel R.Q. (2018): Flavonoids as Th1/Th2 cytokines immunomodulators: A systematic review of studies on animal models. Phytomedicine, 44, 74–84.

Grzegorczyk-Karolak I., Kuzma L., Wysokinska H. (2015): Study on the chemical composition and antioxidant activity of extracts from shoot culture and regenerated plants of Scutellaria altissima L. Acta Physiologiae Plantarum, 37, 1736.

Karabegovic I., Nikolova M., Velickovic D., Stojicevic S., Lazic M. (2011): Comparison of antioxidant and antimicrobial activities of methanolic extracts of the Artemisia sp. recovered by different extraction techniques. Chinese Journal of Chemical Engineering, 19, 504–511.

Khan A.U., Gilani A.H. (2009): Antispasmodic and bronchodilator activities of Artemisia vulgaris are mediated through dual blockade of muscarinic receptors and calcium influx. Journal of Ethnopharmacology, 126, 480–486.

Koretzky G.A. (2010): Multiple roles of CD4 and CD8 in T cell activation. Journal of Immunology, 185, 2643–2644.

Lans C., Turner N. (2011): Organic parasite control for poultry and rabbits in British Columbia, Canada. Journal of Ethnobiology and Ethnomedicine, 7, 21.

Liu N., Ru Y.J., Li F.D., Cowieson A.J. (2008): Effect of diet containing phytate and phytase on the activity and messenger ribonucleic acid expression of carbohydrase and transporter in chickens. Journal of Animal Science, 86, 3432–3439.

Melguizo-Melguizo D., Diaz-de-Cerio E., Quirantes-Pine R., Svarc-Gajic J., Segura-Carretero A. (2014): The potential of Artemisia vulgaris leaves as a source of antioxidant phenolic compounds. Journal of Functional Foods, 10, 192–200.

Oyedemi S.O., Coopoosamy R.M. (2015): Preliminary studies on the antibacterial and antioxidative potentials of hydroalcoholic extract from the whole parts of Artemisia vulgaris L. International Journal of Pharmacology, 11, 561–569.

Pandey A.K., Singh P. (2017): The genus Artemisia: A 2012–2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. Medicines (Basel), 4, 68.

Pandey B.P., Thapa R., Upreti A. (2017): Chemical composition, antioxidant and antibacterial activities of essential oil and methanol extract of Artemisia vulgaris and Gaultheria fragrantissima collected from Nepal. Asian Pacific Journal of Tropical Medicine, 10, 952–959.

Sun Y., Tsao R., Chen F., Li H., Peng H., Jiang L., Chen Y., Deng Z. (2017): The phenolic profiles of Radix Tetrastigma after solid phase extraction (SPE) and their antitumor effects and antioxidant activities in H22 tumor-bearing mice. Food and Function, 8, 4014–4027.

Zhang L.Y. (2016): Feed Analysis and Quality Test Technology. Version 4. China Agricultural University Press, Beijing, China. (in Chinese)

Zhigzhitzhapova S.V., Radnaeva L.D., Gao Q. (2016): Chemical composition of volatile organic compounds of Artemisia vulgaris L. (Asteraceae) from the Qinghai–Tibet Plateau. Industrial Crops and Products, 83, 462–469.

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