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Effect of exogenous enzymes and *Salix babylonica* extract or their combination on haematological parameters in growing lambs*

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ABSTRACT

The aim of this study was to compare the use of exogenous enzyme preparations (EZ) and/or *Salix babylonica* extract (SB) or their combination as feed additives on some haematological parameters in growing lambs. Twenty Suffolk lambs of 6 to 8-months-old with 24±0.3 kg body weight were used in the study. Lambs were divided into 4 groups of 5 animals each in a completely randomized design and the treatments were: 1.control: fed a basal diet of concentrate (30%) and maize silage (70%); 2. EZ: fed the basal diet plus 10 g of enzyme; 3. SB: fed the basal diet plus 30 ml of *S. babylonica* extract, and 4. EZSB: fed the basal diet plus 10 g enzyme and 30 ml of *S. babylonica* extract. Lambs were housed in individual cages and the experiment was conducted for 60 days. The SB was given orally while the EZ was mixed with a small amount of the concentrate and maize silage and was offered *ad libitum*. Blood samples were collected from each animal on days 0, 15, 30, 45 and 60 of

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experiment and analysed for haematological parameters. The treatments of EZ, SB or EZSB did not affect any of the measured blood parameters. Day of sampling modified concentrations of red blood cells (P=0.001; linear effect), haematocrit (P=0.01; quadratic effect), haemoglobin (P=0.01; linear effect), mean corpuscular volume (P=0.01; linear effect), monocytes (P=0.004; quadratic effect) and plasma protein (P=0.0002; linear effect). It could be concluded that *Salix babylonica* extract, exogenous enzymes and their combination as feed additives had not a negative effects on the blood parameters measured and therefore on the health of the lambs.

KEY WORDS: *Salix babylonica* extract, exogenous enzymes, haemotological parameters, lambs

INTRODUCTION

The cost of feeding livestock accounts for more than 50% of the total production costs. Use of feed additives such as pre- and probiotics, ionophores, enzymes, and fodder shrubs and trees extracts have been used recently mainly in ruminant nutrition (Salem et al., 2006, 2010; Chung et al., 2012).

Commercial exogenous enzymes provide the benefits of reducing the anti-nutritional factors in dietary components to improve digestibility (Salem et al., 2012). Some enzyme formulations increased dry matter intake, *in vivo* fibre digestibility, and milk production of dairy cows (Gado et al., 2009, 2011). However, effects of enzyme additives vary depending on the type of animals (energy requirement of the animal and level of feeding), diet composition, enzyme formulation, dose rate, and delivery method of enzyme products (Chung et al., 2012).

Plant extracts rich in secondary compounds have been shown to have positive effects on daily gain, voluntary feed intake, milk production and ruminal fermentation parameters (Salem et al., 2011b), besides having a protective effect on the protein in the rumen to promote duodenal absorption, minimizes the excretion of nitrogen, modify the acetate to propionate ratio in rumen fluid and decrease parasitic load (Athanasiadou and Kyriazakis, 2004; Salem et al., 2010; Jiménez et al., 2011). Although, the use of plant extract could be limit by their secondary compound concentrations, consumption of large amounts of tannins or saponins (Athanasiadou and Kyriazakis, 2004) may have a direct haemolytic effect and may even cause death. Moreover, long-term feeding of plants rich in secondary compounds may have detrimental effects on animal health and even cause death (Mahgoub et al., 2008). Increasing the cost of commercial feed additives (i.e., exogenous enzymes) provide the opportunity for researchers to search for other alternative feed additive such as plant extract.

It was hypothesized that using the exogenous enzymes alone or in combination with tree extracts would have a synergetic effect and infer health benefits to the lambs. Therefore, the aim of this study was to compare the use of exogenous enzymes and *S. babylonica* extract or their combination as feed additives on haematological parameters in growing lambs.

MATERIAL AND METHODS

This study was conducted at the experimental farm of the Faculty of Veterinary Medicine of the Autonomous University of Mexico State. The handling of animals was done according to international bioethical standards and NOM-062-ZOO-1999.

Animals and treatments

Twenty Suffolk lambs of 6 to 8-months-old with 24±0.3 kg body weight (BW) were used in the study. The lambs were housed in individual cages (1.5×1.5 m) in a completely randomized design and the experiment was conducted for 60 days. After 2 weeks of adaptation to the basal diet of 70% maize silage and 30% commercial concentrate (Purina®, Cuautitlan, Mexico) formulated according to NRC (1985) nutrient requirements; the lambs were weighed and randomly distributed into 4 groups of 5 animals each. The treatments comprised: 1. control: lambs were fed the basal diet of concentrate and maize silage only, 2. EZ: lambs were fed the basal diet plus 10 g of exogenous enzyme preparations (ZADO®, Cairo, Egypt), 3. SB: lambs were fed the basal diet plus 30 ml of *Salix babylonica* extract, and 4. EZSB: lambs were fed the basal diet plus 10 g exogenous enzymes and 30 ml of *S. babylonica* extract. The daily dose of SB was given orally before the morning feeding, while the EZ was fed to the lambs mixed with a small amount of the concentrate (200 g) for 20 min and then mixed with the rest of the concentrate and was offered for 1 h before feeding the maize silage *ad libitum* for the rest of the day. The chemical composition of the basal diets is presented in Table 1.

Table 1. Chemical composition of the basal diet (commercial concentrate and maize silage) and levels of secondary metabolites in the *Salix babylonica* extract, g/kg DM

Indices	Concentrate	Maize silage	*Salix babylonica* extract
Chemical composition			
crude protein	147.3	106.2	
ether extract	119.8	85.2	
neutral detergent fibre	160.4	444.8	
acid detergent fibre	27.7	111.2	
acid detergent lignin	7.6	18.0	
Secondary metabolites			
total phenolics			16.4
saponins			5.4
aqueous fraction[1]			76.3

[1] aqueous fraction: lectins, polypeptides, starch (Cowan, 1999)

ZADO® is a powdered multi-enzyme commercially available feed additive product produced from *Ruminococcus flavefaciens* by the Academy of Scientific Research and Technology in Egypt (Patent No. 22155, Cairo, Egypt). Prior to this work, the enzyme mixture was assayed for several enzymatic activities, and it was found to contain (per gram of enzyme preparation) 7.1 unit of endoglucanase, 2.3 unit of xylanase, 61.5 unit of α-amylase and 29.2 unit of protease activity.

To make the silage, whole maize plants (at medium stage i.e., 70% moisture content) were chopped into 1-2 cm pieces using a forage chopper and the silage made immediately in a flat 10-ton silo. After two months, the use of the silage was initiated for feeding experimental animals as well as the other animals of the experimental farm of the faculty.

Preparation of extract

The *S. babylonica* extract was prepared as described by Salem et al. (2011b). Briefly, fresh leaves of *S. babylonica* were collected randomly from several young and mature trees (minimum 5 different trees) in autumn and were chopped (1-2 cm) and immediately extracted in the proportion of 1 g leaf per 8 ml of solvent mixture. The mixture of solvents contained 10 ml methanol (99.8/100, analytical grade, Fermont®, Monterrey, Mexico), 10 ml ethanol (99/100, analytical grade, Fermont®, Monterrey, Mexico) and 80 ml distilled water. Leaves were soaked and incubated in solvent in the laboratory at 25-30°C for 48-72 h in closed flasks. After incubation, all flasks were incubated in a water bath at 39°C for one h and then immediately filtered and the filtrate collected and stored at 4°C for further use.

Sampling and measurements

Five millitres of peripheral blood was collected from each lamb by jugular venipuncture into EDTA vacutainer tubes (BD tube, Monterrey, Mexico) on days 0, 15, 30, 45 and 60 of the experiment. Samples of both concentrate and silage were also collected during the experimental period and stored at -20°C for later chemical analysis.

Two samples of the concentrate, silage and SB extract were collected weekly during the 60 days of experiment. Each sample type (i.e., concentrate, silage or extract) were pooled and stored for further analysis.

Sample analysis

Blood samples were analysed for red blood cell count (RBC), the differential white cell count: neutrophils, lymphocytes, basophils, eosinophils and monocytes,

packed cell volume (PCV), haemoglobin (HGB), mean corpuscular volume (MCV), mean cell haemoglobin concentration (MHC), white blood cell count (WBC), and plasma protein concentration. Haemoglobin concentration was determined using the cyanmethaemoglobin method. Haematocrit was determined by micro haematocrit technique. Erythrocyte and total leucocytes and differential leucocyte counts were determined using the haemocytometer method. Total plasma proteins were determined using a refractometer (Archer and Jeffcott, 1977). Mean MCV and MHC were calculated to diagnose any type of anaemia by taking into account the values of erythrocytes, haemoglobin and haematocrit predetermined:

$$\text{MCV } (\mu m^3/\text{erythrocyte}) = \frac{\text{haematocrit (\%)} \times 10}{\text{total erythrocyte}}$$

$$\text{MHC (\%)} = \frac{\text{haematocrit (g/dl)} \times 10}{\text{haematocrit}}$$

Samples of concentrate and silage were analysed for DM, ash, nitrogen (N), and ether extract (EE) according to AOAC (1997). The neutral detergent fibre (NDF; Van Soest et al., 1991), acid detergent fibre (ADF) and lignin (AOAC, 1997) were analysed using an ANKOM 200 Fibre Analyser Unit (ANKOM Technology Corporation, Macedon, NY, USA). The NDF was assayed without use of α-amylase but with sodium sulphite in the NDF. Both NDF and ADF are expressed without residual ash.

Plant secondary metabolites were determined using ten millitres of extract liquor and fractionated by funnel separation with a double volume of ethyl acetate (99.7/100, analytical grade, Fermont®, Monterrey, Mexico) to determine total phenolics by drying and quantifying the total phenolics layer in the funnel. After total phenolics separation, a double volume of n-butanol (99.9/100, analytical grade, Fermont®, Monterrey, Mexico) was added to fractionate the saponins (Ahmed et al., 1990). The remaining solution was considered to be the aqueous fraction that has the other secondary metabolites such lectins, polypeptides and starch (Cowan, 1999) (Table 1).

Endoglucanase activity was assayed by liberating glucose from carboxymethyl cellulose, which was determined calorimetrically using alkaline copper reagent as described by Robyt and Whelan (1972). One unit of endoglucanase catalyzes the liberation of 1 mmol of glucose per min from sodium carboxymethyl cellulose at 40°C and pH 4.5. Furthermore, α-amylase was assayed by its ability to produce reducing groups from starch, which were measured by the reduction of 3,5-dinitrosalicylic acid (Bernfeld, 1955). One unit of α-amylase catalyzes the liberation of 1 mmol of reducing groups per min from soluble starch at

25°C and pH 6.0, calculated as maltose equivalents. Protease activity was determined by the hydrolysis of dimethyl casein (DMC) and the liberated amino acids were determined using 2,4,6-trinitrobenzene sulphonic acid (Lin et al., 1969). One DMC-U catalyzes the cleavage of 1 mmol of peptide bond per min from DMC at 25°C and pH 7.0 expressed in terms of newly formed terminal amino groups. Xylanase catalyzes the hydrolysis of xylan from oat spelt, and the reducing groups liberated were determined using alkaline copper reagent (Robyt and Whelan, 1972). One unit catalyzes the liberation of 1 mmol reducing groups per h from xylan at 37°C and pH 5.5, expressed as xylose equivalents.

Statistical analyses

Data of the haematological parameters was analysed using the MIXED procedure of SAS (2002) with repeated measures (Littell et al., 1998). The structure of the variance-covariance error matrix employed was unstructured, based on Bayesian criteria observed with several alternative structures. Terms in the model were diet (i.e., control, SB, EZ, EZSB), days of sampling (i.e., 0, 15, 30, 45 and 60 of the experiment) and linear, quadratic and cubic effects were determined. The repeated term was sampling days, with lamb within diet the subject. Results reported in Tables and in text are least square means of fixed effects with their corresponding standard errors. Test of simple effects were used to partition (slice) interaction effects by diet in order to test effects of period separately for each diet (SAS, 2002). Significant differences between treatment means and time were assessed using the Tukey procedure at $P<0.05$ level.

RESULTS

The treatments of EZ, SB or EZSB did not affect any of the measured blood parameters while the day of sampling had a significant impact on some parameters (Table 2). However, red blood cells were increased by 20% ($P=0.001$; linear effect) on day 60, while haemoglobin was increased ($P=0.01$; linear effect) on days 0, 30 and 45 compared with the days 15 and 60 of the experiment. The values of MCV were decreased ($P=0.015$; linear effect) on day 60, while monocytes were increased at days 30 and 45 ($P=0.004$; quadratic effect) compared with others days. Haematocrit was increased ($P=0.02$; quadratic effect) on day 30 and 45.

Table 2. Effect of exogenou s enzyme preparation (EZ) and *S. babylonica* (SB) extract and their combination (EZSB) on haematological parameters in growing lambs after 0, 15, 30, 45, and 60 days of the experiment (n=5 lambs)

Indices	RBC	HMT	HMG	MCV	MHC	WBC	SGN	LYM	MON	BAS	EOS	PSP
Treatment												
control	8.17	0.296	98.4	37.7	331.8	8.71	3.23	5.22	0.027	0.018	0.11	67.12
EZ	8.03	0.286	95.1	35.7	331.9	7.91	2.79	4.94	0.100	0.017	0.08	63.09
SB	8.06	0.296	98.2	37.0	331.8	9.50	3.37	5.82	0.000	0.015	0.10	65.48
EZSB	7.84	0.288	95.4	37.2	331.6	9.28	3.43	5.56	0.022	0.029	0.12	62.84
SEM	0.343	0.0090	3.00	1.26	0.22	0.493	0.301	0.408	0.0180	0.010	0.016	1.293
P value	0.924	0.795	0.790	0.793	0.718	0.146	0.477	0.469	0.220	0.861	0.792	0.097
Day of experiment												
D0	7.77^{c}	0.295^{b}	98.0^{a}	38.4^{a}	331.7	8.84	3.10	5.56	0.006^{c}	0.020	0.11	66.71^{a}
D15	7.24^{c}	0.282^{c}	93.5^{b}	38.9^{a}	331.7	9.44	3.86	5.24	0.000^{c}	0.036	0.17	63.00^{c}
D30	7.56^{c}	0.296^{a}	98.3^{a}	39.3^{a}	331.9	9.03	3.07	5.73	0.070^{a}	0.004	0.13	64.05^{b}
D45	8.19^{b}	0.300^{a}	99.6^{a}	36.6^{b}	331.5	8.33	2.99	5.15	0.120^{a}	0.005	0.09	64.25^{b}
D60	9.36^{a}	0.285^{b}	94.5^{b}	31.0^{c}	332.1	8.62	3.01	5.26	0.000^{c}	0.023	0.09	65.15^{a}
SEM	0.264	0.0056	1.87	1.25	0.23	0.377	0.244	0.286	0.0191	0.010	0.017	0.834
P values												
linear	0.001	0.415	0.013	0.015	0.526	0.076	0.062	0.859	0.688	0.558	0.582	0.001
quadratic	0.893	0.021	0.348	0.649	0.493	0.001	0.102	0.005	0.004	0.739	0.088	0.385
cubic	0.926	0.501	0.841	0.633	0.266	0.334	0.329	0.926	0.583	0.557	0.410	0.350

RBC - red blood cells (x 10^{12}/l); HMT - haematocrit (l/l); HMG - haemoglobin (g/l); MCV - mean corpuscular volume (fi); MHC - mean cell haemoglobin concentration (g/l); WBC - white blood cells (x10^{9}/l); SGN - segmented neutrophils (x10^{9}/l); LYM - lymphocytes (x10^{9}/l); MON - monocytes (x10^{9}/l); BAS - basophils; EOS - eosinophils; PSP - plasma protein (g/dl); ^{a,b,c} different superscripts following means within the same column indicate differences at $P<0.05$; SEM - standard error of the mean

Plasma protein was increased (P=0.001; linear effect) on days 0 and 60 continued to days 30 and 45, but it was decreased on day 30 of the experiment. No significant differences were observed in MHC, blood cells, segmented neutrophils, lymphocytes, basophils and eosinophils among days of sampling during the experiment.

DISCUSSION

In this study, none of the treatments of EZ, SB or EZSB had any effect on blood parameters measured as an indicator for animal health. It has been shown that the use of EZ improves utilization of nutrients in the feed through its effect on their degradation and absorption. This effect was not demonstrated on blood parameters and is consistent with Salem et al. (2011a) who showed that the lambs that consumed EZ treatment did not have signs of disease with the daily addition of 10 g/lamb/day of ZADO® and increased nutrient digestibilities and average daily gain in sheep and goat. In the present study, white blood cells did not differ (P=0.146) among treatments that could be very important results for the role of these cells in immune response innate and acquired as phagocytic cells, antigen presenting cells and cytokine-producing cells (Provenza and Villalba, 2010). These results may be confirm the validity to use the SB extract as feed additive instead of the EZ preparation taking in account the other effects on animal performance. The present results of no impact of treatments on blood parameters is an important finding in the case of those lambs that consumed extract of *S. babylonica* because they contain secondary compounds which could be expected to have a detrimental effect on cellular components and protein in blood mainly because of the presence of tannins and saponins (Khalil and El-Adawy, 1994). It is known that saponins have a haemolytic activity when it was used in high concentration and may cause death to animals by the affinity of the aglycone saponins with cell membrane cholesterol (sterol) which forms a complex and increase in cellular permeability with subsequent loss of haemoglobin (Wang et al., 2007). In the present study, the dose used of secondary compounds in the extract was low therefore we expected that animals consuming extract of *S. babylonica* would increase red blood cells and also have a high amount of circulating haemoglobin in blood which did not happen and the haemoglobin values are within the range for lamb of similar age. Mahgoub et al. (2008) used unconventional feed high in phenolics and condensed tannins in sheep to evaluate their effect on the clinical profile of the animals, and they noted that a negative effect on haematocrit (Mahgoub et al., 2008). These authors also assumed that the detrimental effect of the secondary compound was dependent on the animal species that consume secondary metabolites (Provenza and Villalba, 2010).

Time of sampling had effects on red blood cells, haematocrit, haemoglobin, MCV, monocytes and plasma protein. This may be because ruminants have the ability to adapt to the consumption of plants with high content of secondary compounds such as *S. babylonica*, or EZ addition that improved ruminal microorganisms activities with increasing the time of feeding diets with EZ until 60 days (Salem et al., 2011b). Other haematological parameters such as white blood cells, segmented neutrophils, lymphocytes, basophils and eosinophils were not affected by the sampling time. This result is contradictory because *S. babylonica* secondary compounds have been shown to have effect on white blood cells and their immunostimulatory activity (Provenza and Villalba, 2010). Some studies revealed that ginsenosides and astragalus saponins strengthen the phagocytic function of the reticuloendothelial system, elevate the lymphocyte transformation rate and promote antibody formation (Liu et al., 2011). Saponins have direct effect on the immune system produced a variety of specific antigens and stimulate specific immune response (Weng et al., 2010). Addition of EZ also may be affect on the animal immune response.

CONCLUSIONS

The exogenous enzymes and *Salix babylonica* extract, either individually or in combination had no effect on haematological parameters of lamb as an indicator health status during the 60-day experiment.

REFERENCES

Ahmed V.U., Perveen S., Bano S., 1990. Saponin from the leaves of *Guaiacum officinale*. Phytochemistry 29, 3287-3290

AOAC, 1997.Association of Official Analytical Chemists, Official Methods of Analysis. 16th Edition. Arlington, VA

Archer R.K., Jeffcott L.B., 1977. Comparative Clinical Haemotology. Archer, R.K. & L.B. Jeffcott. (Editors). Internet Bookshop UK Ltd. Gloucestershire, GLS (UK)

Athanasiadou S., Kyriazakis I., 2004. Plant secondary metabolites: antiparasitic effects and their role in ruminant production systems. Proc. Nutr. Soc. 63, 631-639

Bernfeld P., 1955. Amylases, alpha and beta. Methods. Enzymol. 1, 149-158

Chung Y.-H., Zhou M., Holtshausen L., Alexander T.W., McAllister T.A., Guan L.L., Oba M., Beauchemin K.A., 2012. A fibrolytic enzyme additive for lactating Holstein cow diets: Ruminal fermentation, rumen microbial populations, and enteric methane emissions. J. Dairy Sci. 95, 1419-1427

Cowan M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564-582

Gado H.M., Salem A.Z.M., Odongo N.E., Borhami B.E., 2011. Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Anim. Feed Sci. Tech. 165, 131-136

Gado H.M., Salem A.Z.M., Robinson P.H., Hassan M., 2009. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Anim. Feed Sci. Tech.154, 36-46

Jiménez P., Salem A.Z.M., Mejia H.P., González R.M., Albarrán P.B., Rojo R.R., Tinoco J.L., 2011. Influence of individual and mixed extracts of two tree species on *in vitro* gas production kinetics of a high concentrate diet fed to growing lambs. Livest. Sci. 136, 192-200

Khalil A.H., El-Adawy T.A., 1994. Isolation, identification and toxicity of saponin from different legumes. Food Chem. 50, 197-201

Lin Y., Gary E., Robert E., 1969. Action of proteolytic enzymes on N,N-dimethyl proteins. Basis for a micro-assay for proteolytic enzymes. J. Biol. Chem. 244, 789-793

Littell R.C., Henry P.R., Ammerman C.B., 1998. Statistical analysis of repeated measures data using SAS procedures. J. Anim. Sci. 76, 1216-1231

Liu H.W., Tong J., Zhou D.W., 2011. Utilization of chinese herbal feed additives in animal production. Agric. Sci. China 10, 1262-1272

Mahgoub O., Kadim I.T., Tageldin M.H., Al Marzooqi W.S., Khalaf S.Q., Amnbu Ali A., 2008. Clinical Profile of sheep fed non- conventional feeds containing phenols and condensed tannins. Small Ruminant Res. 78, 115-122

NRC, 1985. Nutrient Requirements of Domestic Animals. National Research Council.Washington, DC

Provenza F.D., Villalba J.J., 2010. The role of natural plant products in modulating the immune system: an adaptable approach for combating disease in grazing animals. Small Ruminant Res. 89, 131-139

Robyt J.F., Whelan W.J., 1972. Reducing valuemethods for maltodextrins: 1. Chain length dependence of alkaline 3,5-dinitrosalicylate and chain length independence of alkaline copper. Anal. Biochem. 45, 510-516

SAS, 2002. SAS User´s Guide: Statistics. Ver 9.0. SAS Institute. Cary, NC

Salem A.Z.M., El-Adawy M., Gado H., Camacho L.M., Ronquillo M., Alsersy H., Borhami B., 2011a. Effects of exogenous enzymes on nutrients digestibility and growth performance in sheep and goats. Trop. Subtrop. Agroecosyst. 14, 867-874

Salem A.Z.M., Hassan A.A., Khalil M.S., Gado H.M., Alsersy H., Simbaya J., 2012. Effects of sun-drying and exogenous enzymes on nutrients intake, digestibility and nitrogen utilization in sheep fed *Atriplex halimus* foliages. Anim. Feed Sci. Tech. 171, 128-135

Salem A.Z.M., Olivares M., Lopez S., Gonzalez-Ronquillo M., Rojo R., Camacho L.M., Cerrillo S.M.A., Mejia H.P., 2011b. Effect of natural extracts of *Salix babylonica* and *Leucaena leucocephala* on nutrient digestibility and growth performance of lambs. Anim. Feed Sci. Tech. 170, 27-34

Salem A.Z.M., Robinson P.H., López S., Gohar Y.M., Rojo R., Tinoco J.L., 2010. Sensitivity of sheep intestinal lactic acid bacteria to secondary compounds extracted from *Acacia saligna* leaves. Anim. Feed Sci. Tech. 161, 85-93

Salem A.Z.M., Salem M.Z.M., El-Adawy M.M., Robinson P.H., 2006. Nutritive evaluations of some browse tree foliages during the dry season: Secondary compounds, feed intake and *in vivo* digestibility in sheep and goats. Anim. Feed Sci. Tech. 127, 251-267

Van Soest P.J., Robertson J.B., Lewis B.A., 1991. Methods of dietary fiber, neutral detergent fiber and non-polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583-3597

Wang Y., Zhang Y., Zhu Z., Zhu S., Li Y., Li M., Yu B., 2007. Exploration of the correlation between the structure, hemolytic activity, and cytotoxicity of steroid saponins. Bioorgan. Med. Chem. 15, 2528-2532

Weng A., Jenett-Siems K., Schmieder P., Bachran D., Bachran C., Görick C., Thakur M., Fuchs H., Melzig F.M., 2010. A convenient method for saponin isolation in tumour therapy. J. Chromatogr. 878, 713-718