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Original Research Article

Effect of Methanol Leaf Extract of *Lophira lanceolata (Ochnaceae)* on Blood Glucose and Serum Proteins Concentration in Doxorubicin Induced Rats

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Abstract

Serum proteins play important roles in the body and their increase or decrease signify a health state. *Lophira lanceolata* is a medicinal plant used as a remedy for many diseases. This study was aimed at investigating the effect of methanol leaf extract of *Lophira lanceolata* (Ochnaceae) on blood glucose and serum proteins concentration in doxorubicin diseased rats. Adult albino rats of both sexes weighing 125-330 g were divided into 9 treatment groups of 6 animals per group and were treated for 28days with 2.5 mg/kg doxorubicin, 100, 200 and 400 mg/kg of the extract, 1.2 mg/kg aspirin and 1 ml/ kg Tween 80. Blood was withdrawn from each group before and after treatment and was assayed. Results obtained were analyzed statistically using one-way ANOVA. The extract alone caused a significant ($p < 0.05$, $p < 0.001$) dose dependent increase in serum total proteins, albumin and globulin at all dose level when compared to the standard. A decrease in the blood sugar concentration of the group treated with combination of the extract and doxorubicin was also observed. These findings suggest that methanol extract of the leaf of *Lophira lanceolata* has blood sugar lowering effect and can cause increase in serum protein concentrations.

Keywords: Blood-sugar, Doxorubicin, *Lophira lanceolata,* Serum proteins

INTRODUCTION

Proteins are the major components of blood serum and plasma, possessing a wide range of important physiological functions like maintaining acid-base balance. In the circulatory system they serve as carriers of biological molecules such as vitamins, lipids and minerals and are also involved in cellular activity and immune system (Tothova et al., 2016). Albumins and globulins are the major serum proteins and their concentrations are important indicator of health state. A number of factors such as dehydration, drugs, hepatic dysfunction, glomerulonephritis can cause an increase or decrease in the concentrations of these proteins, resulting in disease processes (Pieper et al. 2003). Doxorubicin (Dox) an anthracycline antineoplastic drug used for the treatment of breast cancer and other neoplastic diseases (Aryal et al., 2014), has been associated with cardiotoxicity occurring due to the production of reactive oxygen species (ROS) which caused protein carbonylation with subsequent decrease in levels of major serum proteins (Ichikawa et al., 2014).

Numerous plants and herbs have been reported to possess free radical scavenging and antioxidant properties. Thus, they are useful in the management and prevention of cardiovascular diseases and other ailments involved with the generation of ROS (Alasalvar et al., 2006). The mechanism of their antioxidant activity can be either by directly attacking the reactive oxygen species or by enhancing natural defense of cells. *Lophira lanceolata*, one of the species of *Lophira* belonging to the Ochnaceae family is a multipurpose tree, used in traditional medicine to treat several illnesses due to its radical scavenging activity (Oussou et al., 2016). The decoction of the fresh leaves has been used orally to treat headaches, dysentery, diarrhea, cough, abdominal

pains and hypertension (Oussou et al., 2016). A concoction of the root has been used by women against menstrual pain, intestinal troubles and malaria and pulmonary diseases. In southern Nigeria, the bark of the root has been used to manage gastrointestinal problems and yellow fever (Onyeto et al., 2014). Previous studies on the leaves of *L. lanceolata* have shown anti-hypertensive, anti-diarrheal, anti-plasmodial and antioxidant effects of the water and methanol extracts (Igboeli et al., 2015). Various studies have been carried out on the antioxidant activity of *Lophira lanceolata*, but few studies are available regarding its effect on serum concentration of proteins and blood sugar level. The study was designed to investigate the effect of *Lophira lanceolata* on serum protein and blood sugar level of doxorubicin diseased rats.

MATERIALS AND METHODS

Animals

Adult Wister rats of both sexes (125-330 g) obtained from the animal house of the Department of Pharmacology and Toxicology, University of Nigeria Nsukka, were used for the investigation. The animals were kept in cages at room temperature (25 °C) and naturally illuminated environment of 12:12 h dark/light cycle. They were fed on standard diet and had water *ad libitum*. The animals were acclimatized for 7 days before initiation of the experiment.

Chemicals and standards

The chemicals used where of analytical and international standard. They include, Tween 80 (Guangdong Guanghua Sci-Tech Co. Ltd. China), 70 % methanol (Sigma Chemical Company Ltd. USA), distilled water (Energy and Resources Centre, U.N.N), Vasoprin tablet (75 mg aspirin) (Juhel Nigeria Limited), Doxorubicin Hydrocloride Injection powder for reconstitution 50 mg (zuvius Lifesciences pvt Ltd. Mumbai), Biurett reagent (Randox Laboratories Ltd. London), and Bromocresol Green solution (Randox Laboratories Ltd. London).

Equipment

The equipment utilized were Electrical weighing balance (B. Bran Scientific and Instruments Co. England), UV-Visible spectrophotometer (Easy-Way Medical England 752W, England), Milling Machine(Lab mill serial No.4745, Christy and Norris Ltd. England), Large extraction vessels(B. Bran Scientific and Instruments Co. England), Sigma 6-16 centrifuge (Sciquip Ltd, Uk), Accu-check Blood Glucose Meter (Roche Diagnostics, Germany), automatic pipette (Super-fit Equipment, Ames), micro-capillary tube and test tubes (Marienfeld, Germany).

Plant collection and identification

The young leaves of the plant *Lophira lanceolata* were collected from Kogi State, Nigeria in July 2020. The plant was identified and authenticated by Mr. Alfred Ozioko, a taxonomist and staff of the International Centre for Ethnomedicine and Drug development (Inter CED) Nsukka Enugu State, Nigeria. A dried voucher specimen was preserved at the Pharmacognosy Herbarium, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka (specimen number: PCG/UNN/0311) thereafter.

Preparation of plant extract

The fresh leaves collected were properly air-dried at room temperature then milled into fine powder (5.05 kg). The powdered leaves were then subjected to cold maceration using 22.5 L of 70 % methanol and was shaken constantly for 72 h. The mixture was then filtered using Whatman No.1 filter paper. The resultant filtrate was concentrated at room temperature to a yield of 6.55 % (w/w).

Phytochemicals analysis and acute toxicity test

Preliminary phytochemical studies were carried out on the extract for the presence of alkaloids, tannins, saponins, terpenes, flavonoids, oils, glycosides, steroids, and carbohydrates.

The safety of the extract was orally evaluated by determining its LD_{50} (Onyeto et al., 2014).

Drug treatment procedure

The method employed was described by Paramasivan et al. (2012). After acclimatization for seven days, the animals were divided into nine groups of six rats per group.

Group I: Doxorubicin 2.5 mg/kg body weight IP on days 1, 7, 14, 21 and 28

Group II: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21 and 28) + Methanol leaf Extract of *L. lanceolata* (100 mg/kg body weight for 28days).

Group III: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21 and 28) + Methanol leaf Extract of *L. lanceolata* (200 mg/kg body weight for 28days).

Group IV: Doxorubicin (2.5 mg/kg body weight IP on days 1, 7, 14, 21 and 28) + Methanol leaf Extract of *L.*

lanceolata (400 mg/kg body weight for 28days).

Group V: Doxorubicin (2.5 mg/kg body weight IP on days 1,7,14, 21 and 28) + Aspirin (1.2 mg/kg body weight for 28days)

Group VI: Methanol Leaf Extracts of *L. lanceolata* only (100 mg/kg body weight for 28days).

Group VII: Control (Tween 80, 1 ml/kg body weight for 28days).

Group VIII: Methanol Leaf Extracts of *L. lanceolata* only (200 mg/kg body weight for 28days).

Group IX: Methanol Leaf Extracts of *L. lanceolata* only (400 mg/kg body weight for 28days).

Before and after the experimental period, blood was drawn from the animals by inserting a capillary tube into para-orbital venous complex to obtain both pre-treatment and post-treatment results. The animals were sacrificed under light chloroform anesthesia. Assays were carried out to determine the following.

Estimation of blood sugar level

The blood sugar level of the Wistar albino rats were determined using the method of Kermani et al. (2017). Accu-check Active machine was used for the determination of the blood glucose level.

Estimation of serum total protein

The most widely used method for measuring serum protein is the biuret reaction. The principle of this reaction is that serum proteins react with copper sulphate in sodium hydroxide to form a violet biuret complex. The intensity of the violet color was measured using a DRE 3000 HACH spectrophotometer and is proportional to the concentration of protein (Ekam and Udosen., 2012).

Estimation of serum albumin

Albumin is generally measured by a dye-binding technique that utilizes the ability of albumin to form a stable complex with bromocresol green dye. The absorbance of the samples and of the standard was measured against reagent blank at 546 nm, and temperature of 37 °C. These tubes and their contents were mixed and incubated for 90 min at 37 °C. Estimation of albumin level (g/dl) was obtained using a DRE 3000 HACH spectrophotometer (Ekam and Udosen., 2012).

Estimation of serum globulin

Since, bromocresol green (BCG) – albumin complex absorbs light at a different wavelength from the unbound dye, the method may overestimate albumin by binding to other proteins. Hence, the total globulin fraction is generally determined by subtracting the albumin fraction from the total protein fraction. (Ekan and Udosen., 2012).

Statistical analysis

The results obtained were expressed as mean ± S.E.M. The data were analyzed using one-way ANOVA and DUNNET post hoc (Graph pad prism version 5 software). $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

RESULTS

Phytochemicals analysis and acute toxicity test

The percentage yield of the crude extract calculated as percentage of the starting material was 6.55 % w/w. The methanol leaf extract of L lanceolata gave positive test for flavonoid, alkaloid, glycoside, saponins, terpenoids, reducing sugar, oils and carbohydrate (Table 1). After 24 h of both the first and second phases of the acute toxicity test, there was no mortality recorded in the mice upon oral administration even at doses as high as 5000 mg/kg.

Effect of *L. lanceolata* extract on blood glucose, serum total protein, serum albumin, and serum globulin levels

The extract (200 and 400 mg/kg) caused decrease in fasting blood sugar of the animals on day 28. This decrease however was not significant ($p > 0.05$) when compared to the control. The effect of the extract on blood glucose could be compared to that of the standard drug 75 mg Aspirin (1.2 mg/kg). The extract at all dose level, significantly ($p < 0.05$) elevated the serum protein levels of rats treated with the extract alone when compared to the control. Both extract and standard drug did not cause any significant changes to the total protein levels of diseased rats. The extract at all dose level did not cause an increase in serum albumin in the doxorubicin diseased rats. While the extract alone at 200 and 400 mg/kg concentrations caused a significant ($p < 0.05$, $p < 0.01$) increase in serum albumin compared to the control. There was marked elevation of serum globulin in animals treated with the extract alone. This increase was dose independent and significant ($p < 0.05$) when compared to the control. The standard drug and extract had no significant elevation of serum globulin in the doxorubicin diseased rats when compared to the control (Table 2 and 3).

Table 1. Phytochemical constituents of *Lophira lanceolata*

Phytoconstituents	ME
Alkaloids	+
Glycoside	++
Carbohydrates	+
Tannins	-
Renins	-
Steroids	-
Reducing sugars	+
Saponins	+
Oil	++
Flavonoids	+++
Terpenoids	+
Proteins	-
pH	Acidic

KEY: (-) = absent; (+) = mildly present; (++) = moderately present; (+++) = highly present. ME = methanol extract

Table 2. Baseline results for the fasting blood sugar, serum total protein, serum albumin and globulin of the untreated

Treatment groups	Dose (mg/kg)	Fasting blood sugar(mg/dl)	Serum total protein(g/l)	Serum albumin(g/l)	Serum globulin(g/l)
Tween 80	-	72.50±2.99	6.81±0.22	3.71±0.06	3.07±0.20
Dox	2.5	74.50±4.02	6.96±0.12	3.63±0.06	3.33±0.12
ME/Dox	100/2.5	70.50±3.30	6.63±0.22	3.51±0.15	3.12±0.08
ME/Dox	200/2.5	79.67±4.52	6.85±0.09	3.75±0.13*	3.10±0.17
ME/Dox	400/2.5	82.50±3.64	6.78±0.13	3.51±0.04	3.27±0.12
Asp/Dox	1.2/2.5	71.33±5.27	6.78±0.09	3.83±0.13**	2.95±0.19
ME	100/2.5	67.50±2.34	6.79±0.11	3.73±0.12*	3.06±0.15
ME	200/2.5	69.00±2.54	6.56±0.18	3.44±0.12	3.12±0.15
ME	400/2.5	74.50±3.50	7.02±0.18	6.32±0.14	3.31±0.23

ME = methanol extract, Dox = doxorubicin, Asp = Aspirin

Table 3. Effect of the methanol leaf extract of *Lophira lanceolata* on blood glucose, serum total proteins, serum albumin and serum globulin of doxorubicin and non-doxorubicin diseased rats

Treatment groups	Dose (mg/kg)	Fasting blood sugar(mg/dl)	Serum total protein(g/l)	Serum albumin(g/l)	Serum globulin(g/l)
Tween 80	-	66.00±2.80	8.20±0.22	3.82±0.08	4.38±0.23
Dox	2.5	74.17±3.52	6.71±0.30	3.22±0.13	3.54±0.26
ME/Dox	100/2.5	79.80±3.38	6.52±0.12	3.18±0.12	3.34±0.20
ME/Dox	200/2.5	69.83±2.10	6.74±0.24	3.22±0.14	3.51±0.12
ME/Dox	400/2.5	69.75±2.05	6.58±0.29	3.07±0.22	3.51±0.07
Asp/Dox	1.2/2.5	69.40±2.40	6.50±0.15	3.04±0.10	3.47±0.18
ME	100	65.50±2.55	8.00±0.14***	3.70±0.10*	4.30±0.21*
ME	200	77.60±3.80	7.98±0.11***	3.81±0.33**	4.17±0.14
ME	400	71.80±3.78	7.93±0.04***	3.68±0.06	4.25±0.02

Results expressed as Mean ± SEM, n = 6, $p^* < 0.05$, ** $p < 0.01$, ***$p < 0.001$ significant compared to negative control. ME = methanol extract, Dox = doxorubicin, Asp = Aspirin

DISCUSSION

In this study, the effect of methanol leaf extract of *L. lanceolata* on blood glucose concentration, serum total proteins, albumin and globulin of doxorubicin diseased rats was assesed. The results revealed that the extract has blood sugar lowering effect and also caused an increase in serum proteins concentration. In the acute

toxicity test, there was no deaths in the rats within 24 h up to 5000 mg/kg oral dose. This suggest that the extract is relatively nontoxic acutely (Onyeto et al., 2014).

Phytochemical analysis of the methanol extract of *Lophira lanceolata* revealed the presence of flavonoids, terpenoids, alkaloids, glycosides, carbohydrates, oils and reducing sugars. This result is in accordance with those obtained by Onyeto et al (2019). Ethanol, chloroform and petroleum ether fractions of the leaves of *Lophira lanceolata* has showed the presence of phenols, flavonoids, glycosides, tannins, carbohydrates and sugars on phytochemical analysis (Audu et al., 2007). As reported in the literature, there is a high relationship between the total phenol and total flavonoid contents and the antioxidant effect of medicinal plants (Sharififar et al., 2009). In addition to flavonoids and phenolic compounds, some of the alkaloids, saponins and triterpenoids are reported to also possess antioxidant activity making the plant material a rich source of antioxidants (Khan et al., 2012).

Administration of doxorubicin alone intraperitoneally on days 1,7,21 and 28 caused observable damage to the cardiovascular system in the experimental animals. The cellular damage and disease progression seen in doxorubicin induced cardiotoxicity occur as a result of protein carbonylation (Rao, 2013 and Brioschi et al., 2012) and increased carbonylation of these proteins is associated with a significant reduction in corresponding protein levels in serum (Dickey et al., 2013). In this study methanol extract of *Lophira lanceolata* was seen to cause a dose independent increase in serum concentrations of total serum protein, serum albumin and globulin. However, a significant increase in the serum proteins were observed in the group treated with the extract alone compared to the control group. This effect might be as a result of the antioxidant effect of the leaf (Onyeto et al., 2014). It has been reported that bioactive fractions of various medicinal plants having free radical scavenging and antioxidant properties are useful for the prevention and treatment of cardiovascular diseases (Oussou et al., 2016). This could therefore justify the use of *Lophira lanceolata* in the treatment of hypertension as reported by (Kouakou et al., 2013). The mechanism which can be by either directly preventing the binding of transition metal ion catalysts, by causing decomposition of peroxides, prevention of continued abstraction of hydrogen, increased reductive capacity and radical scavenging or by enhancing natural defense of cells (Onyeto et al., 2014). Albumin and globulin play important physiological and pathological roles in the body. Albumin amongst other functions, plays an important role in maintaining homeostasis, in transport of substances, and acts as a free-radical scavenger (Hankins, 2006) while the main functions of globulin is to defend the host against pathological damage, assist in the restoration of homeostasis and in the regulation of different stages of inflammation (Petersen et al., 2004).

Elevated protein carbonylation due to oxidative stress have been detected in both type-I and type-II diabetes. Treatment of carbonyl stress in diabetes offers new therapeutic approaches, including redox modulation, reactive carbonyl detoxification and inhibition of oxidative stress (Dane-Donne et al., 2003). The methanol leaf extract of the plant material was seen to cause a decrease in the blood sugar concentration of the doxorubicin diseased rats. Although an increase in the blood sugar level of the group treated with the extract alone was observed, this effect might be attributed to the negative feedback mechanism of the heart. Methanol extract of the leaves of *Lophira lanceolata* has shown to possess antihyperglycemic activity (Houndjo et al., 2017). This could be ascribed to the natural antioxidants agents it contains (Oussou et al., 2016). Antioxidants such flavonoids, terpenoids and phenols possess the ability to protect cells and tissues from damages caused by free radical and reactive oxygen species (Oussou et al., 2016).

CONCLUSION

The outcome of this investigation revealed that, the methanol leaf extract of *L. lanceolata* is safe and has effect in reduction of blood sugar, elevation of serum concentrations of total proteins, albumin and globulin. Nevertheless, further investigations are needed as to the actual concentration that will cause a reversal of the decrease in serum proteins seen with doxorubicin induced toxicity. This knowledge may lead to the discovery of remedies for cardiotoxicity induced by doxorubicin and other agents involved in protein carbonylation.

REFERENCES

Alasalvar C, Karamac MR, Amarowicz and Shahidi F (2006). Antioxidant and antiradical activities in extracts of hazelnut kernel (*Corylus avellana L.)* and hazelnut green leafy cover. J. Agric. Food Chem. 54(13): 4826-4832.

Aryal B, J. Jeong, Rao VO (2014). Doxorubicin-induced carbonylation and degradation of Cardiac myosin binding protein C promote cardiotoxicity. Proceedings of the National Academy of Science 111(5):2011-2016.

Audu SA, I. Mohammed, Kaita HA (2007). Phytochemical screening of leaves *Lophira lanceolata* (Ochnaceae). Life Science Journal 4(4):75-79. ISSN: 1097-8135.

Brioschi M, Polvani G, Fratto P, Parolari A, Agostoni P, E. Tremoli, Banfi C (2012). Redox proteomics identification of oxidatively modified myocardial proteins in human heart failure: implications for protein function. PLoS ONE 7(5): e 35841.

Dalle-Donne I, Giustarini D, Colombo R, R. Rossi, Milzani A (2003). Protein Carbonylation in human diseases. Trends in molecular medicine 9(4): 169-176.

Dicky SJ, Gonzalez Y, Aryal B, Mog S, Nakamura JA, Redon EC, Baxa U, Rosen E, Chen G, Jacek Z, Parekh P, Mason PK, Joseph

J, Kalynaraman B, Bonner W, Herman E, E. Shacter, Rao VA (2013). Mito-tempol and dexrazoxane exhibit cardioprotective and chemotherapeutic effects through specific protein oxidation and autophagy in a syngeneic breast tumor preclinical model. PLoS ONE 8(8): e70575.

Ekam VS, Udosen EO (2012). Total Protein, albumin and globulin levels following the administration of activity directed fractions of *Vernonia Amygdalina* during acetaminophen induced hepatotoxicity in wistar albino rats. Glo. J. Pure and Appl. Sci. 18(1&2): 25-29.

Hankins J (2006). The role of albumin in fluid and electrolyte balance. Journal of Infusion Nursing 29(5):260-265.

Heart EA, Karandrea S, Liang X, Balke ME, Beringer PA, Bobczynski EM, Burgos DZ, T. Richardson, Gray JP (2016). Mechanisms of doxorubicin toxicity in pancreatic β-cells. Toxicological Sciences 152(2): 395-405.

Houndjo CF, Agbodjogbe W, Assogba FM, Kohoude JM, Ayedoun MA, Dansou PH, M. Moudachirou, Gbenou JD (2017). Comparative study of antihyperglycemic activity of aqueous extracts from the leaves of *Bridelia ferruginea, Lophira lanceolata* and *Oxytenanthera abyssinica*, with their mixture. Int. J. Curr. Res. Chem. Pharm. Sci. 4(11):22-33.

Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga-Prasad SV, Mutharasan RK, TJ Naik, Ardehali H (2014). Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J. Clin. Investigation 124(2):617-630.

Igboeli N, Onyeto CA, Okorie AN, Mbaoji FN, IA Nwabunike, Alagboso DI (2015). Antidiarrheal activity of methanol leaf extract of *Lophira lanceolata Tiegh* (Ochnaceae). Merit Res. J. Environ. Sci. Toxicol. 3(4): 059-064.

Kermani SK, Khatony A, Jalali R, M. Rezaei, Abdi A (2017). Accuracy and precision of measured blood sugar values by three glucometers compared to the standard technique. J. Clin. Diagnostic Res. 11(4): OC05-OC08.

Khan RA, Khan MR, S. Sahreen, Ahmed M (2012). Assessment of flavonoids contents and in vitro antioxidant activity of *Launaea procumbens*. Chemistry Central Journal 6(1):43

Kouakou KL, Bleyere NM, Oussou NJ-B, Konan BA, Amonkan KA, Abo KJ-C, A.P. Yapo, Ehile EE (2013). Effects of leaf decoction from *Lophira lanceolata Tiegh.* Ex Keay (Ochnaceae) on arterial blood pressure and electrocardiogram in anesthetized rabbits. Pharma Innovation 2(9): 66-73.

Onyeto CA, Akah AA, Nworu SC, Okoye TC, Okorie NA, Mbaoji FN, Nwabunike IA, N. Okumah, Okpara O (2014). Anti-plasmodial and antioxidant activities of methanol extract of the fresh leaf of *Lophira lanceolate* (Ochnaceae). African Journal of Biotechnology. 13(16): 1731-1738.

Onyeto CA, SA Ihim, Akah PA (2019). Hepatoprotective effect of the methanol leaf extract of Lophira lanceolata (Ochnaceae): An experimental study. Merit Res. J. Med. Med. Sci. 7(3): 100-105.

Oussou NJ-B, Asiedu-Gyekye JI, Yapo FA, N'guessan BB, Amoateng P, Kouakou KL, KI Asante, Ehile EE (2016). In-vitro scavenging activity and acute toxicity of methanol leaves extract and fractions of *Lophira lanceolata* TIEGH. EX KEAY(Ochinaceae) in rats. Int. J. Phytomed. 8(3): 411-421.

Petersen HH, JP Nielsen, Heegaard PM (2004). Application of acute phase protein measurements in veterinary clinical chemistry. Veterinary Research 35(2):163-187.

Pieper R, Gatlin CL, Makusky AJ, Russo PS, Schatz CR, Miller SS, Su Q, McGrath AM, Estock MA, Parmar PP, Zhao M, Huang ST, Zhou J, Wang F, Esquer-Blasco R, Anderson NL, J. Taylor, Steiner S (2003). The human serum proteome: display of nearly 3700 chromatographically separated protein spots on two-dimensional electrophoresis gels and identification of 325 distinct proteins. Proteomics 3, 1345-1364.

Rao VA (2013). Iron chelators with Topoisomerase-inhibitory activity and their anticancer applications. *Anitoxidants & Redox Signaling* 18(8):930-955.

Sharififar F, G. Dehghn-Nudeh, Mirtajaldini M (2009). Major flavonoids with antioxidant activity from *Teucrium polium L.* Food Chemistry 112**:** 885-888.

Tothova C, O. Nagy, Kovac G (2016). Serum proteins and their diagnostic utility in veterinary medicine: a review. Veterinarian Medicina 61 (9): 475-496.