

Gas Chromatography and Mass Spectroscopy of *Juniperus Phoenix* Stem Bark Extract and Its Influence on the Haemato-Biochemical Values of Growing Rabbits

Alagbe, J.O

Department of Animal Nutrition and Biochemistry Sumita Research Institute, Gujarat India

dralagbe@outlook.com

Orcid number: 0000-0003-0853- 6144

Article Information

Received: October 13, 2022

Accepted: October 22, 2022

Published: October 28, 2022

Keywords

Juniperus phoenice, rabbits, phytochemicals, blood, gas chromatography, mass spectroscopy

ABSTRACT

Juniperus phoenice stem bark have been source of wide array of bioactive compounds with endless therapeutic properties. This study evaluated the gas chromatography and mass spectroscopy of *Juniperus phoenice* stem bark extract and its influence on the haemato-biochemical values of growing rabbits. Gas chromatography and mass spectroscopy of *Juniperus phoenice* stem bark extract revealed the presence of 37 phytoconstituents with varying concentrations with total aggregate of 95.28 %. 40 – 6 weeks growing male rabbits (Newzealand white × chinchilla) weighing 456 ± 8.03 were randomly assigned to 4 groups (A, B, C and D) of 10 animals which was further divided into 5 replicates consisting of 2 rabbits each in a completely randomized design. Basal diet according to the nutrient requirement of rabbits outlined by NRC (1977). Animals in group A was fed basal diet with 0 % *Juniperus phoenice* stem bark extract (JPSB) while B, C and D were fed basal diet with JPSB at 3 mL, 6 mL and 9 mL once daily. It was observed that rabbits in group D fed 9 mL/day had a significantly ($P < 0.05$) higher pack cell volume, haemoglobin, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentrations, mean corpuscular volume, white blood cells and their differentials compared to the other treatment except basophil count were not significantly ($P > 0.05$) influenced by the treatments. Similarly, serum biochemical indices values were topmost in G3 (6 mL/day) and G4 (9 mL/day), midway in G2 (3 mL/day) and lowest in G1 (0 mL/day). Creatinine, urea and total bilirubin count were not significantly ($P > 0.05$) different among the treatments. It was concluded that JPSB has potential pharmaceutical properties and can be fed to growing rabbits up to 9 mL/day without jeopardizing the health of animals.

Introduction

As consumers around the world demand for antibiotic free animal husbandry, medicinal plants are predicted to have a promising future in animal nutrition due to their broad range of efficacies and to their effects on sustainability and safety. Plant extracts contains several bioactive ingredients that may promote feed efficiency, nutrient utilization as well as good health in livestock (IPP, 2001). One of the holistic approach to multidrug resistance is the use of medicinal plant especially *Juniperus phoenice*.

Juniperus phoenice is a member of the family Cupressaceae which consist of over 70 species which is widely distributed throughout the tropical and subtropical regions of the world (Bekhech *et al.*, 2001). Extracts from *Juniperus phoenice* stem, leaves and roots have been used for the

treatment of various diseases such as gastrointestinal diseases, skin infections, haemorrhoids, cough, fever, inflammation, headache and asthma (Cavaleiro *et al.*, 2001). Phytochemical evaluation of the stem bark revealed the presence of a wide range of bioactive compounds including flavonoids (Rezzi *et al.*, 2011), phenols (Fouad *et al.*, 2012), alkaloids (Afifi *et al.*, 1995), steroids (Dob *et al.*, 2005), terpenoids and saponins (El-Sawi *et al.*, 2007). Pharmacological investigation revealed that *Juniperus phoenice* leaves, stem and root have antimicrobial, antioxidant, anti-inflammatory, cytotoxic, hepato-protective, antimutagenic, antiparasitic, antiproliferative, antifungal, antiviral, antihelminthic, analgesics, antipyretic, cardio-protective, immune-modulatory and antiprotozoal properties.

Aqueous extract of *Juniperus phoenice* stem bark were accounted to possess α -cubebene, α -longipinene, γ -terpinene, β -caryophyllene, β -elemenone, β -santalene, D-limonene, α -humulene, 3-methylmannoside, α -amorphene and γ -selinene when subjected to gas chromatography and mass spectrometry analysis (Jean *et al.*, 2006). *In vitro* studies have shown that aqueous and methanolic stem bark extract from *Juniperus phoenice* were capable of reducing the activities of pathogenic bacteria's including; *Pseudomonas putida*, *Ralstonia picketti*, *Salmonella spp*, *Staphylococcus spp*, *Klebsiella spp*, *Micrococcus luteus*, *Escherichia coli*, *Citrobacter freundii*, *Bacillus subtilis*, *Neisseria spp*, *Enterobacter spp*, *Erwinia spp*, *Brucella spp*, *Acinetobacter spp*, *Alcaligenes pacificus*, *Kocuria varians* and *Erwinia spp* keeping the intestinal flora in a natural balance and also preventing the entry of toxic substances into the blood of animals (Lin *et al.*, 1999; Moreno *et al.*, 1994; Zgoda and Porter, 2001).

Most *in vivo* investigations have shown that plant extracts exerts a positive effects on the blood parameters of rabbits. Oloruntola *et al.* (2016) reported that a significant ($P<0.05$) increase in the haematological parameters (haemoglobin, pack cell volume, red blood cells and leucocytes) of growing rabbits fed *Alchomea cordifolia*. Similar result was observed by Alipour *et al.* (2015); Oloruntola *et al.* (2018a) who recorded a notable difference ($P<0.05$) in haematological and serum biochemical indices of animals fed plant extract derived from thyme and neem, pawpaw and bamboo leaf meal respectively.

Due to the unlimited bioactive compounds in *Juniperus phoenice* stem bark extract and its possibility as a top solution to the increasing cases of antimicrobial resistance. The aim of this study is to evaluate the gas chromatography and mass spectroscopy of *Juniperus phoenice* stem bark extract and its influence on the haemato-biochemical values of growing rabbits.

Materials and methods

Experimental site, plant material and extraction procedure

The experiment was carried out at Sumitra Research Institute Gujarat, India (23° 13'N 72°41'E) in the month of January to March, 2021. *Juniperus phoenice* stem bark were collected from Khavda village in Gujarat. Taxonomic identification was carried out by Dr. Singh Amit at the Department of Biological Sciences of Sumitra institute. Fresh stem bark of *Juniperus phoenice* was chopped into pieces using a kitchen knife, air dried for 15 days to preserve the secondary metabolites in the material. It was grinded into powder using an electric blender for 10 minutes.

The powdered stem bark was extracted with 70 % ethanol over a 48 hours period at a room temperature of 25°C with soxhlet apparatus. The mixture was filtered with Whatman filter paper, thereafter, it was dried in a rotary evaporator (Model RS-100-PRO, China) with dimension (D×P×H mm) – 465 × 457 × 583 mm, speed range (20-280 rpm), stroke (150 mm) and temperature

(RT – 180 °C). The extract was stored in a white labelled sterile container and kept in the refrigerator at 4 °C before it was sent to the laboratory for additional examination.

Gas chromatography and mass spectroscopy of *Juniperus phoenice* stem bark extract (JPSB)

The bioactive chemicals in *Juniperus phoenice* stem bark (JPSB) was carried out using gas chromatography coupled to mass spectroscopy (GC-MS) model 6800 N gas chromatography coupled to 5189 F mass spectroscopy from Sukray auto sampler. The GC had the following technical specifications; inlet temperature 450 °C, pressure range (100 psi \pm 0.001 psi), split mode (split/splitless, max split ratio: 1000:1) and column oven working temperature (+4 °C~ 450 °C) while MS specifications; EI source ionization energy (5 eV – 250 eV), mass range (1.5 – 1000 amu), ion source temperature (100 -300 °C), stability (\pm 0.10 amu/48 hours), scan rate (up to 1000 amu/s) and detector (high energy dynode electron multiplier).

Reagents required include: solid calcium chloride, liquid nitrogen, 100 mM ethylene diamine tetra acetate (EDTA) with 7.5 pH, helium 5.0 and 10 mL headspace screw cap vials.

2 μ L of *Juniperus phoenice* stem bark extract (JPSB) is injected via the inlet it goes into the column with non-polar coating to the detector before retention and peaks were generated all other procedures were strictly adhered according to the manufacturer's recommendation.

Experimental animal management, diet formulation and design

40 – 6 weeks growing male rabbits (Newzealand white \times chinchilla) weighing 456 ± 8.03 were sourced from Sumitra Teaching and Research Farm, Gujarat. Animals were transferred early in the morning (7 am) and kept in a specially constructed battery cages with dimension 95 cm \times 70 cm \times 35 cm (Length \times width \times height) in a semi closed housing system were placed on two weeks adaptation period during which they were fed only the basal diet (Table 1) and water *ad libitum* with other preventive treatment (deworming). After adaptation, rabbits were shared into 4 groups (A, B, C and D) of 10 animals which was further divided into 5 replicates consisting of 2 rabbits each in a completely randomized design. Biosecurity and other management practices strictly maintained throughout the experimental period (10 weeks).

Feed ingredients such as; yellow corn, brewer's dry grain, wheat offal, soya meal, palm oil, mineral and vitamin premix, toxin binder, methionine, lysine and salt were properly mixed together to formulate a basal diet according to the nutrient requirement of rabbits outlined by NRC (1977). Animals were also fed twice daily (7:30 am) and (3:00 pm) in the morning and afternoon respectively. Rabbits in group A was fed basal diet with 0 % *Juniperus phoenice* stem bark extract (JPSB) while B, C and D were fed basal diet with JPSB at 3 mL, 6 mL and 9 mL once daily.

Proximate analysis of experimental diet

Proximate analysis of experimental diet was done using near infra-red (NIR) Fibertec™ 8000 automatic feed analyzer with technical specifications; 1000 – 2000 nm wave length range, frequency (60/70 Hz), power consumption of 60W and resolution VIS (15 nm). 100 g of feed sample is placed into the sample cup and placed on the spectrometer (automatic detector) product is selected using an icon displayed on the monitor while progress is displayed within 15-30 seconds to show the quality of the product.

Determination of haematological parameters

At the end of the study 2 mL of blood samples were collected from the marginal ear vein of 5 randomly selected rabbits per group for haematological analysis. Blood samples for haematology were placed in bottles containing ethylene diamine tetra acetate (EDTA) and were analyzed using

Mindray BC-5390 auto-haematology analyzer. Samples were pre-diluted with reagents according the manufacturers specification and placed in a sample processing unit, run and auto scan sample identification and thereafter monitor and report analyzer errors. Analytical principles of red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration measurement (Electrical impedance method), haemoglobin concentrations (Calorimetric method), white blood cell count, lymphocytes, monocytes, basophils, eosinophils and neutrophils (Chemical dye flow cytometry laser scatter and differential scatter gram measurements).

Determination of serum biochemical indices

Sample of blood for serum analysis (2 mL) was transferred into bottles without EDTA using semi-auto blood chemistry analysis (Model CCL-WP21E, China). The parameters analyzed are; albumin, globulin, urea, creatinine, total bilirubin, cholesterol, high density lipoprotein, low density lipoprotein, sodium ion, chloride ion, bicarbonate and potassium ion.

Calibration of the machine is the first stage of analysis this is done using appropriate reagent recommended by the manufacturer. Thereafter blood samples were arranged on the sample processing unit. Progress on the arrangements is displayed on the monitor (data processing unit) before selecting the parameters, outcome on the experimental results was transmitted on the data managing unit.

Statistical analysis

Results were subjected one-way analysis of variance (ANOVA) using SPSS V.23.0 software (SPSS Institute Inc., Cary, NC) Duncan's test was used to determine the differences in the mean values ($P < 0.05$).

Table 1: Chemical composition of experimental diets

Constituents	Portion (Kg)
Yellow corn	30.00
Wheat offal	28.60
Brewers dry grain	11.00
Soy bean meal	18.00
Di-calcium phosphate	6.00
Palm oil	4.05
Salt	0.30
**Mineral & Vitamin premix	0.25
Toxin binder	0.20
Methionine	0.15
Lysine	0.15
Total	100.0
Calculated analysis (g/kg DM)	
Dry matter	890.46
Crude protein	162.00
Ash	13.57
Crude fibre	146.08
Ether extract	9.50
Metabolizable energy (kcal/kg)	2766.1

** Vitamin-mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU;

cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B12, 12.5 µg; riboflavin, 5.5 mg; Ca panthothenate, 11 mg; niacin, 53.3 mg; choline chloride, 1,020 mg; folic acid, 0.75 mg; biotin, 0.25 mg; delquin, 125 mg; DL-Met, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; and NaCl, 2,500 mg

Results and Discussions

Secondary metabolites of *Juniperus phoenice* stem bark extract using GC-MS

Chromatography is a fundamental technique used to separate complex mixtures with pharmaceutical and biomedical properties (Duran *et al.*, 2015). They also aid in the discovery of innovative and novel compounds of therapeutic potentials (Abdel *et al.*, 2012; Adewale *et al.*, 2021). Secondary metabolites are bioactive compounds produced by plants via metabolic pathways (shikimic acid pathways, mevalonic pathways and melonic acid pathways) and they are sources of medications traditionally used for the treatment of human and animal diseases (Shittu and Alagbe, 2020; Alagbe *et al.*, 2022). For instance, flavonoids aids in the inhibition of microbial growth (preservative) (Nychas, 1995; Alagbe, 2019) and could also act as an antimicrobial (Akintayo and Alagbe, 2020; Agubosi *et al.*, 2022), anti-inflammatory, antioxidant (Marina and Mihailo, 2011), antiviral, cytotoxic and immunostimulatory functions (Mughal *et al.*, 1996; Alagbe, 2019; Agubosi *et al.*, 2022). Alkaloids are organic compounds characterized by a nitrogen atom in a heterocyclic ring which have considerable anti-proliferative, analgesics (Schmidt *et al.*, 2006; Guida *et al.*, 1999), antifungal, hepato-protective and miracicidal properties (Keskes *et al.*, 2017; Alagbe and Grace, 2019). Phenolic acid are bioactive compounds with antioxidant (Das and Maulik, 1995; Al-Qirim *et al.*, 2002, anti-cancer, antibacterial and anti-proliferative, hypocholesteromic, antiviral and hypolipidemic activities (Saba *et al.*, 2012; Daglia, 2012). Several pharmacological benefits have also be reported in terpenoids, tannins, saponins and oxalates (Firn, 2010; Edeoga *et al.*, 2005). However, higher concentration of these secondary metabolites can be detrimental to the health of animals. Higher concentration of oxalates in the body of animals can limit calcium absorption (Omokore and Alagbe, 2019).

GC-MS analysis of *Juniperus phoenice* stem bark extract used in this experiment (Table 2) display the presence of 37 phytoconstituents with varying concentrations. The highest and lowest chemical compounds recorded in JPSB are α -pinene and heptacosane respectively. Other chemical compounds present in JPSB contain wide array of potential pharmaceutical properties. The presence of α -pinene implies that the plant parts (leaf, stem bark and roots) can be used traditionally for the treatment of respiratory disease, skin infection, cough, sexually transmitted diseases, obesity, snake bites and measles (Karaman *et al.*, 2003). The GC-MS result is in consonance with the reports of Moreno *et al.* (1998) but conversely to the findings of Jean *et al.* (2006). This disparity could be linked to environmental differences, species, quality of kit used in analysis as well as extraction procedure used (Alagbe, 2019).

Table 2: Secondary metabolites of *Juniperus phoenice* stem bark extract using GC-MS

Name of compounds	% Area	Mol. Formula	Mole. weight g/mol
β -elemenone	13.66	C ₁₅ H ₂₂ O	218
α -humulene	8.01	C ₁₅ H ₂₄	204.357

α -cubebene	3.50	C ₁₅ H ₂₄	204.35
γ -terpinene	2.53	C ₁₀ H ₁₆	136.23
α -longipinene	2.00	C ₁₅ H ₂₄	204.35
γ -eudesmol	0.77	C ₁₅ H ₂₆ O	222.37
β -caryophyllene	1.94	C ₁₅ H ₂₂ O	204.357
β -santalene	1.05	C ₁₅ H ₂₄	204.35
α -pinene	20.31	C ₁₀ H ₁₆	136.238
β -citrylideneethanol	2.50	C ₁₀ H ₂₀ O	156.269
Torreyol- α -cadinol	0.01	C ₁₅ H ₂₆ O	222.3663
D-limonene	9.04	C ₁₀ H ₁₆	136.23
Benzene (2-methoxy-2-peopenyl)	0.06	C ₁₀ H ₁₂ O	148
3-Methylmannoside	0.003	C ₇ H ₁₄ O ₆	194
Elemol	2.01	C ₁₅ H ₂₆ O	222.37
α -Amorphene	1.09	C ₁₅ H ₂₄	204.35
Cedrene	0.01	C ₁₅ H ₂₄	204.357
γ -Selinene	0.07	C ₁₅ H ₂₄	204.35
9,12-Octadecadienoic acid	0.28	C ₁₈ H ₃₂ O ₂	280
δ -Cadinene	6.66	C ₁₅ H ₂₄	204.35
Trans-Caryophyllene	0.22	C ₁₅ H ₂₄	204.35
Thymol	2.05	C ₁₀ H ₁₄ O	150.221
Camphor	0.09	C ₁₀ H ₁₆ O ₂	152.237
Hexyl isovalerate	0.17	C ₁₁ H ₂₂ O ₂	186.29
Pinocarveol	0.22	C ₁₀ H ₁₆ O	152.237
Allo-Ocimene	0.03	C ₁₀ H ₁₆	136.23
Isospathulenol	0.41	C ₁₅ H ₂₄ O	220.35
Eicosane	0.72	C ₂₀ H ₄₂	282.5
Heptacosane	0.01	C ₂₇ H ₅₆	380.7
Germacrene	0.05	C ₁₅ H ₂₄	204.35
3-methoxy-p-cymene	0.004	C ₁₈ H ₂₁ NO ₃	299.4
4-methyl-2,3-hexadien-1-ol	0.39	C ₇ H ₁₂ O	112.17
1,2-Cyclopentanedione	0.04	C ₅ H ₆ O ₂	98.10
3-Allyl-6-methoxyphenol	0.003	C ₁₀ H ₁₂ O ₂	164.201
2-Methyl-4-vinylphenol	0.02	C ₉ H ₁₀ O	134.17
Glycidol stearate	0.26	C ₂₁ H ₄₀ O ₃	340.5
Myrcene	15.09	C ₁₀ H ₁₆	136.238
Aggregate	95.28	-	-

Influence of *Juniperus phoenice* stem bark extract on the haematological values of growing rabbits

Table 3 displays the influence of *Juniperus phoenice* stem bark extract on the haematological values of growing rabbits. The haematological parameters examined includes; red blood cell, pack cell volume, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration, white blood cell, leucocytes, neutrophils, monocytes, eosinophils, basophils and

lymphocytes which have a lower and upper limit values of 4.73 – 5.81 ($\times 10^6/\mu\text{L}$), 28.92 – 37.04 (%), 9.11 – 12.86 (g/dL), 51.87 – 73.91 fl, 18.82 – 32.20 (pg), 29.15 – 45.88 (%), 6.88 – 11.22 ($\times 10^3/\mu\text{L}$), 25.60 – 37.00 (%), 5.44 - 9.13 (%), 0.93 – 1.11 %, 2.05 – 2.31 % and 27.11 – 37.22 % respectively. All the parameters were significantly ($P < 0.05$) affected by the treatment except for basophil values ($P > 0.05$). Red blood cell, leucocytes, hemoglobin, pack cell volume, lymphocytes, monocytes, mean corpuscular volume and mean corpuscular haemoglobin concentrations were top most in G3 and G4, midway in G2 and under most in G1 ($P < 0.05$). Conversely, neutrophils and eosinophils count were highest in G2, G3, G4 and lowest in G1 ($P < 0.05$). Nonetheless, all values were within the referenced ranges of normal haematological parameters of growing rabbits (Brockus, 2011; Abdel-Azeem *et al.*, 2010). According to Research Animal Resources (2009), pack cell volume is the volume of red blood cell's packed at the bottom of an haematocrit tube when blood is centrifuged. Lower value of pack cell volume below 20 % is a sign of anaemia or cirrhosis of liver while an elevated level could be an indication of dehydration or polycythemia (Meredith, 2014). Red blood cell (RBC) plays an important role in gaseous exchange i.e. delivers oxygen from lungs to the tissues and gets rid of carbon dioxide from the tissues to the lungs (Hewitt *et al.*, 1989). It also performs buffering activities in the blood (Poole, 1987). Physiological factors (age, sex, breed and high temperatures) and pathological factors (anaemia and polycythemia) can affect RBC count (Ozkan *et al.*, 2012). Haemoglobin is the protein molecule or red blood pigment found in the erythrocytes which carries oxygen from the lungs to the tissues of animals (Hewitt *et al.*, 1989). Values of pack cell volume, haemoglobin, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin within the normal physiological range reported in this experiment is a clear sign that feeding *Juniperus phoenice* stem bark extract between 6-9 mL daily had no negative effect on the health of rabbits. Mean corpuscular volume (MCV) is the average volume of a single red blood cell. When is normal the red blood cell is called normocyte, when it increases (macrocyte) and decreases (microcyte) (Lassen and Welser, 2004). Mean corpuscular haemoglobin concentration (MCHC) is the concentration of haemoglobin in one red blood cell. When MCHC is normal the red blood cell is normochromic and decreased level (hypochromic) (Gillet, 1994).

Leucocytes are the body defense systems (antibodies production). They also move to the predilection sites of pathogens and engulf bacteria using pseudopodia via a process called phagocytosis (Chineke *et al.*, 2006; Alagbe *et al.*, 2019). Rabbits in G3 and G4 had the highest value of leucocytes count compared to the other groups ($P < 0.05$) making them less susceptible to disease and infection. An abnormal decrease in leucocytes count is referred to as leucopenia while an increase (leukocytosis) due to infection (Loeb and Quimby, 1989). Eosinophils secrete anti-toxins and are associated with allergies while basophils release histamines for inflammation which dilate the blood vessels (Gillet, 1994). Lymphocytes are responsible for killing pathogenic bacteria's that invade the body of animals while monocytes invade junks and are produced in the bone marrow (Lassen and Welser, 2004).

Tables 3: Influence of *Juniperus phoenice* stem bark extract on the haematological values of growing rabbits

Variables	Group 1	Group 2	Group 3	Group 4	SEM
Pack cell volume (%)	28.92 ^c	31.77 ^b	36.95 ^a	37.04 ^a	1.85
Haemoglobin (g/dL)	9.11 ^c	10.28 ^b	12.49 ^a	12.86 ^a	0.71
Red blood cell ($\times 10^6/\mu\text{L}$)	4.73 ^c	5.05 ^b	5.72 ^a	5.81 ^a	0.25



MCV (fl)	51.87 ^c	63.94 ^b	71.50 ^a	73.91 ^a	0.66
MCH (pg)	18.20 ^c	22.41 ^b	31.82 ^a	32.20 ^a	0.92
MCHC (%)	29.15 ^c	38.73 ^b	45.71 ^a	45.88 ^a	0.35
Leucocytes ($\times 10^3/\mu\text{L}$)	6.88 ^c	9.09 ^b	10.44 ^a	11.22 ^a	0.18
Neutrophils (%)	25.60 ^b	37.10 ^a	37.49 ^a	37.00 ^a	0.97
Eosinophils (%)	0.93 ^b	1.80 ^a	1.06 ^a	1.11 ^a	0.31
Monocytes (%)	5.44 ^c	8.73 ^b	9.09 ^a	9.13 ^a	0.44
Basophils (%)	2.05	2.88	2.74	2.31	0.51
Lymphocytes (%)	27.11 ^c	30.78 ^b	37.59 ^a	37.22 ^a	0.43

SEM: standard error of mean; MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration

^{a,b,c} Means within a row with different manuscripts differ significantly ($P < 0.05$)

Influence of *Juniperus phoenice* stem bark extract on the serum biochemical traits of growing rabbits

Influence of *Juniperus phoenice* stem bark extract on the serum biochemical traits of growing rabbits is displayed in Table 4. The serum biochemical indices evaluated are; total protein, albumin, globulin, α 1-globulin, α 2-globulin, β -globulin, γ -globulin, cholesterol, creatinine, urea, total bilirubin, calcium, phosphorus, potassium, sodium, chloride and bicarbonate which have a lower and upper limit values of 4.79 – 6.02 g/dL, 1.91 – 2.98 g/dL, 2.88 – 3.04 g/dL, 1.78 – 4.33 g/dL, 0.92 – 1.91 g/dL, 1.00 – 2.59 g/dL, 0.88 – 1.93 g/dL, 1.00 – 3.77 Mmol/L, 50.60 – 59.41 Mmol/L, 5.40 – 6.05 Mmol/L, 5.15 – 5.80 Mmol/L, 2.90 – 4.45 Mmol/L, 0.71 – 1.47 Mmol/L, 4.88 – 5.50 Mmol/L, 102.4 – 160.7 Mmol/L, 86.65 – 108.6 Mmol/L and 44.62 – 77.49 Mmol/L respectively. Values of α 1-globulin, α 2-globulin, β -globulin, γ -globulin, sodium, chloride, calcium, potassium, bicarbonate and phosphorus were highest in G3 and G4, midway in G2 and lowest in G1 ($P < 0.05$) contrary to total protein values which were topmost in G4 compared to the other treatments ($P < 0.05$). Urea, creatinine and total bilirubin values were not significantly ($P > 0.05$) influenced by the treatment. Cholesterol values were highest in G1 compared to the other treatments ($P < 0.05$). Albumin are synthesized by the hepatocytes and are capable of maintaining blood volume and body fluid distribution (Tumova *et al.*, 2013). Degradation of albumin provides essential amino acid during malnutrition (Singh *et al.*, 2002). Albumin may be considered as the transport form of essential amino acid from the liver to extra hepatic cells (Loeb and Quimby, 1989). Alpha and beta (α and β) globulins are synthesized in the liver while gamma (γ) globulins are synthesized by plasma cells and β cells of lymphoid tissues (Martinec *et al.*, 2012). Decreased concentration of globulin is a sign of poor or low protein as well as hepatic diseases (Meredith, 2014). Gamma (γ -globulin) are immunoglobulin fractions which aids in neutralization of foreign substances in the body (Martinec *et al.*, 2012). However, all the values were within the range reported for clinically healthy rabbits (Hewitt *et al.*, 1989). Cholesterol serves as a precursor of bile, production of vitamin D and steroid hormones. In this experiment, cholesterol level decreases as the level of *Juniperus phoenice* stem bark extract increased across the group. This shows that *Juniperus phoenice* stem bark extract is capable of reducing the risk of coronary or cardiovascular disease (Brokus, 2011). Urea is a waste product excreted by the kidney and an elevated level in the blood of animals could be as a result of dehydration or high protein in the diet (Archetti *et al.*, 2008). Creatinine is also used as a marker of kidney function (Martinec *et al.*, 2012). The non-

significant ($P>0.05$) differences in creatinine and urea level among the animals implies that there was no renal degeneration (Adewale *et al.*, 2021).

Potassium is a major intracellular cation in the body saddled with the responsibility of contraction of heart, neuromuscular excitability and potassium balance is regulated by the kidney (Frieden, 1984). Increase in potassium plasma above normal level is called Hyperkalemia while a decreased level (Hypokalemia) (Sidhu *et al.*, 2004). Chloride is a major extracellular anion which maintains blood volume, electric neutrality and osmotic pressure (Tajeda *et al.*, 2009). Bicarbonate acts as a buffering compound in the blood to prevent acidosis (Underwood, 1971). However, all the physiological values were within the reference range reported by Abdel-Azeem *et al.* (2010); Martinec *et al.* (2012) on selected blood indicators in different rabbit breeds.

Table 4: Influence of *Juniperus phoenice* stem bark extract on the serum biochemical traits of growing rabbits

Parameters	Group 1	Group 2	Group 3	Group 4	SEM
Total protein (g/dL)	4.79 ^c	5.83 ^b	5.88 ^b	6.02 ^a	0.70
Albumin (g/dL)	1.91 ^c	2.90 ^b	2.95 ^b	2.98 ^a	0.16
Globulin (g/dL)	2.88 ^b	2.93 ^b	2.93 ^b	3.04 ^a	0.25
α 1-globulin (g/dL)	1.78 ^c	3.03 ^b	4.15 ^a	4.33 ^a	0.40
α 2-globulin (g/dL)	0.92 ^c	1.07 ^b	1.88 ^a	1.91 ^a	0.44
β -globulin (g/dL)	1.00 ^c	1.96 ^b	2.22 ^a	2.59 ^a	0.31
γ -globulin (g/dL)	0.88 ^c	1.06 ^b	1.51 ^a	1.93 ^a	0.03
Cholesterol (Mmol/L)	3.77 ^a	1.90 ^b	1.01 ^c	1.00 ^c	0.12
Creatinine (Mmol/L)	59.41	53.05	55.18	50.60	0.60
Urea (Mmol/L)	6.05	5.67	5.45	5.40	0.29
T. Bilirubin (Mmol/L)	5.15	5.80	5.71	5.52	0.22
Calcium (Mmol/L)	2.90 ^c	3.95 ^b	4.24 ^a	4.45 ^a	0.14
Phosphorus (Mmol/L)	0.71 ^c	1.00 ^a	1.25 ^a	1.47 ^a	0.01
Potassium (Mmol/L)	4.88 ^c	5.00 ^b	5.13 ^a	5.50 ^a	0.05
Sodium (Mmol/L)	102.4 ^c	144.9 ^b	156.3 ^b	160.7 ^a	7.11
Chloride (Mmol/L)	86.65 ^c	98.15 ^b	101.2 ^a	108.6 ^a	5.37
Bicarbonate (Mmol/L)	44.62 ^c	67.08 ^b	75.70 ^a	77.49 ^a	2.06

SEM: standard error of mean

^{a,b,c} Means within a row with different manuscripts differ significantly ($P<0.05$)

Conclusion

Juniperus phoenice stem bark extract is a source of wide array of secondary metabolites which could stimulate the animals' body to stimulate the production of antibodies and improved nutrient utilization. The presence of phenolic compounds in *Juniperus phoenice* stem bark extract also makes them capable of preventing diseases in rabbits. It was concluded that *Juniperus phoenice* stem bark extract could be fed to growing rabbits up to 9 mL per day causing any deleterious effect on the health of animals.

References

1. Alagbe, J.O., Agubosi, O.C.P., Oluwafemi, R.A and Gabriel Zakara (2022). Efficacy of *Trichilia monadelpha* stem bark on the growth performance of growing rabbits. *British Journal of Innovation in Science, Research and Development*, 1(2): 10-19.
2. Frieden E (1984). Biochemistry of the essential ultratrace elements. Plenum press, New York
3. Alagbe, J.O., Shittu, M.D., Ramalan, S.N., Tanimomo, K.B and Adekunle, D.A. (2022). Growth performance, semen quality characteristics and hormonal profile of male rabbit bucks fed *Rubia cordifolia* root extracts. *International Journal of Biological Engineering and Agriculture* 1(1): 1-13.
4. Sidhu, P., Gorg, M.L., Morgenstern, P., Vogt, J., Butz, T and Dhawan, D.K (2004). Role of Zinc in regulating the levels of hepatic elements following nickel toxicity in rats. *Biological Trace Elements Research*, 102: 161-172.
5. Jean, B.B., Ange, B., Carlos, C., Ligia, S and Joseph, C. (2006). Chemical variability of *Juniperus oxycedrus* spp and leaf oils from Corsica analyzed by combination of GC, GC-MS. *Favour and Fragrance Journal*, 21: 268-273.
6. Alagbe, J.O., Shittu, M.D and Tanimomo, Babatunde K. (2022). Influence of *Anogeissusleio carpus* stem bark on the fatty acid composition in meat of broiler chickens. *European Journal of Life Safety and Stability* 14(22): 13-22.
7. Abdel-Azeem A.S., Abdel-Azim A.M., Darwish A.A., Omar E.M. (2010). Haematological and biochemical observations in four pure breeds of rabbits and their crosses under Egyptian environmental conditions. *World Rabbit Sci.*, 18: 103-110. doi:10.4995/wrs.2010.18.13.
8. Archetti I., Tittarelli C., Cerioli M., Brivio R., Grilli G., Lavazza A. (2008). Serum chemistry and hematology values in commercial rabbits: preliminary data from industrial farms in northern Italy. In Proc.: 9th World Rabbit Congress, June 10-13, 2008, Verona, Italy, 1147-1151.
9. Brockus C.W. (2011). Erythrocytes. In: Latimer K.S. (Ed.). *Duncan and Prassers's Veterinary Laboratory Medicine: Clinical Patology* (5th ed). Wiley-Blackwell, Chichester, UK, 3-44.
10. Burnett N., Mathura K., Metivier K.S., Holder R.B., Brown G., Campbell M. (2006). An investigation into haematological and serum chemistry parameters of rabbits in Trinidad. *World Rabbit Sci.*, 14: 175-187. doi:10.4995/wrs.2006.556.
11. Chineke C.A., Ologun A.G., Ikeobi C.O.N. (2006). Hematological parameters in rabbit breeds and crosses in humid tropics. *Pak. J. Biol. Sci.*, 9: 2102-2106. doi:10.3923/pjbs.2006.2102.2016.
12. Hewitt C.D., Innes D.J., Savory J., Wills M.R. (1989). Normal biochemical and hematological values in New Zealand White rabbits. *Clin. Chem.*, 35: 1777-1779.
13. Gillet C.S. (1994). Selected drug dosages and clinical reference data. In: Manning P.J., Ringler D.H., Newcomer C.E. (Eds.). *The Biology of the Laboratory Rabbits* (2nd ed). Academic Press, New York, USA, 467-472. doi:10.1016/B978-0-12- 469235-0.50028-2.
14. Alagbe, J.O., Shittu, M.D and Ushie, F.T. (2021). GC-MS analysis of methanolic stem bark extract of *Zollingeriana indigofera*. *Asian Journal of Advances in Research* 11(4): 144-146.
15. Alagbe, J.O (2021). Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Growth Performance and Caecal Microbial Population. *Concept in Dairy and Veterinary Sciences*. 4(2):2021

16. Jeklová E., Leva L., Knotigová P., Faldyna M. (2009). Age-related changes in selected haematology parameters in rabbits. *Res. Vet. Sci.*, 86: 525-528. doi:10.1015/j.rvsc.2008.10.007.
17. Lassen E.D., Weiser G. (2004). Laboratory Technology for Veterinary Medicine. In: Thrall M.A. (Ed.). *Veterinary hematology and clinical chemistry* (1st ed). Lippincott Williams & Wilkins, Philadelphia, USA, 3-38.
18. Loeb W.F., Quimby, F.W. (Eds.). (1989). *The clinical chemistry of laboratory animals* (1st ed). Pergamon Press, New York, USA.
19. Martinec M., Härtlová H., Chodová D., Tůmová E., Fučíková A. (2012). Selected haematological and biochemical indicators in different breeds of rabbits. *Acta Vet. Brno*, 81: 371-375. doi:10.2754/avb201281040371
- Meredith A. 2014. The value of clinical pathology in pet rabbit medicine. *Vet. Rec.*, 174: 552-553. doi:10.1136/vr.g3531
20. Alagbe, J.O (2022). Use of medicinal plants as a panacea to poultry production and food security: A review. *Gospodarka I Innowacje* 22(2022): 1-12.
21. Özkan C., Kaya A., Akgül Y. (2012). Normal values of haematological and some biochemical parameters in serum and urine on New Zealand White rabbits. *World Rabbit Sci.*, 20: 253-259. doi:10.4995/wrs.2012.1229.
22. Karaman, F., Sahin, M., Güllüce, H., Ögütçü, M., Sengül, A and Adıgüzel, S. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology* 85: (2003) 231–235.
23. Tůmová E., Martinec M., Volek Z., Härtlová H., Chodová D., Bízková Z. (2013). A study of growth and some blood parameters in Czech rabbits. *World Rabbit Sci.*, 21: 251-256. doi:10.4995/wrs.2013.1320.
24. Alagbe, J.O., Adedeji, M.O., Habiba, Z., Nwosu, Gloria and Wyedia Dabara Comfort (2021). Physico-chemical properties of *Indigofera zollingeriana* seed oil. *Asian Journal of Advances in Medical Science* 3(4): 306-308.
25. Adewale, A.O., Alagbe, J.O., Adeoye, Adekemi. O. (2021). Dietary Supplementation of *Rauvolfia Vomitoria* Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3): 1-12.
26. Shittu, M.D., Alagbe, J.O., Adejumo, D.O., Ademola, S.G., Abiola, A.O., Samson, B.O and Ushie, F.T. (2021). Productive Performance, Caeca Microbial Population and Immune-Modulatory Activity of Broiler Chicks Fed Different Levels *Sida Acuta* Leaf Extract in Replacement of Antibiotics. *Bioinformatics and Proteomics Open Access Journal* 5(1): 000143.
27. Alagbe, J.O. (2021). *Prosopis africana* stem bark as an alternative to antibiotic feed additives in broiler chicks diets: Performance and Carcass characteristics. *Journal of Multidimensional Research and Reviews*, 2(1): 64-77.
28. Alagbe J.O, Shittu MD, Ajagbe Adekunle David. (2020). *Albizia lebbeck* stem bark aqueous extract as alternative to antibiotic feed additives in broiler chicks' diets: performance and nutrient retention. *International Journal of Zoology and Animal Biology*, 3(5):000237.

29. Tejada-Jimenez, M., Galvan, A., Fernandez, E and Lamas A. (2009). Homeostasis of the micronutrients Ni, Mo and Cl with specific biochemical functions. *Current Opinion in Plant Biology*, 12: 358-363
30. Shittu, M.D and Alagbe, J.O. (2020). Phyto-nutritional profiles of broom weed (*Sida acuta*) leaf extract. *International Journal of Integrated Education*. 3(11): 119-124.
31. Akintayo Balogun Omolere. M and Alagbe, J.O (2020). Probiotics and medicinal plants in poultry nutrition: A review. *United International Journal for Research and Technology*, 2(1): 7-13.
32. Alagbe, J.O. (2020). Effect of dietary supplementation of *Cymbopogon Citratus* oil on The Performance and Carcass characteristics of broiler chicks. *European Journal of Biotechnology and Bioscience*. 8(4): 39-45.
33. Alagbe, J.O (2019). Proximate, mineral and phytochemical analysis of *Piliostigma thonningii* stems bark and roots. *International Journal of Biological, Physical and Chemical Studies*, 1(1): 1-7.
34. Alagbe, J.O. (2019). Growth performance and haemato-biochemical parameters of broilers fed different levels of *Parkia biglobosa* leaf extracts. *Academic Journal of Life Sciences*. 5(12): 107 – 115.
35. Alagbe, J.O., Sadiq, M.R., Anaso, E.U and Grace, F.R. (2019). Efficacy of *Albizia lebbeck* seed oil on the growth performance and carcass characteristics of weaner rabbits. *Sumerianz Journal of Agriculture and Veterinary*. 2(12): 116-122.
36. Alagbe, J.O and Grace, F.R. (2019). Effect of *Albizia lebbeck* seed oil dietary supplementation on the haematological and serum biochemical parameters of weaner rabbits. *Sumerianz Journal of Agriculture and Veterinary*. 2(10): 96 -100.
37. Omokore, E.O and Alagbe, J.O. (2019). Efficacy of dried *Phyllanthus amarus* leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. *International Journal of Academic Research and Development*. 4(3): 97-104.
38. Alagbe, J.O. (2019). Growth response and bacteria count of broiler starter given *Delonix regia* leaf extract as a natural alternative to antibiotics. *Food and Nutrition: Current Research*. 2(3): 197 – 203.
39. Degia, M. (2012). Polyphenols as antimicrobial agent. *Current Opinion in Biotechnology*, 23:174-181.
40. Guida, M.P., Casoria, C and Melluso, G. (1999). Preliminary report on antimicrobial activity of *Helichrysum litoreum*. Guss. Boll. Chim Farm, 138(7): 369-373.
41. Firn, R. (2010). Nature's Chemicals: The natural products that shaped our World. Oxford University Press
42. Edeoga, H.O., Okwu, D.E and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7): 685-688.
43. Underwood, E.J (1971). Trace Elements in Human and Animal Nutrition, 3 rd Edition, Academic Press, New York p. 116.
44. Al-Qirim, T.M., Shahwan, M., Zaidi, K.R and Banu, N. (2002). Effect of khat, its constituents and resistant stress on free radical metabolism of rats. *Journal of Ethno pharmacology*, 87: 3-9.
45. Das, D.K and Maulik, N. (1995). Protection against free injury in the heart and cardiac performance in rat. *African Journal of Biotechnology*, 95: 359-388.

46. Saba, A.B., Onakoya, O.M., Oyagbami, A. A. (2012). Hepatoprotective and in vivo antioxidant activities of ethanolic extract of the whole fruit of *Lagenaria breviflora*. Journal of Basic Clinical Physiology and Pharmacology, 23: 27-32.
47. Research Animal Resource [RAR]. (2009). Reference values for laboratory animals: Normal haematological values. RAR Websites, RAR, University of Minnesota. Retrieved from <http://www.ahc.umn.edu/rar/refvalues.html>
48. Poole, T. B. (1987). The UFAW Handbook on the Care and Management of Laboratory and other research Animals (6th ed.). Universities Foundation for Animal Welfare, Longman Scientific and Technical, Harlow, U.K.
49. Özkan, C., Kaya, A and Akgül, Y. (2012). Normal values of haematological and some biochemical parameters in serum and urine of New Zealand White rabbits. World Rabbit Science, 20(4), 253-259.
50. Alagbe, J.O. (2018). Effect of different levels of dried *Delonix regia* seed meal on the performance, haematology and serum biochemistry of growing Grass cutters. *Agricultural Research and Technology Open Access Journal*. 18(4):001-006.
51. Alagbe, J.O. (2019). Performance and haemato-biochemical parameters of weaner rabbits fed diets supplemented with dried water melon (rind) meal. *Journal of Dairy and Veterinary Sciences*, 8(4):001-007.
52. Alagbe, J.O. (2018). Growth and haematological parameters of Japanese quails (*Coturnix coturnix japonica*) fed dried Corn silk- *Polyalthia longifolia* leaf meal mixture. *Pacific International Journal*. 1(3):29-39.
53. Singh, A. S., Pal, D. T., Mandal, B. C., Singh, P and Pathak, N. N. (2002). Studies on Changes in Some of Blood Constituents of Adult Cross-bred Cattle Fed Different Levels of Extracted Rice Bran. *Pakistan Journal of Nutrition*, 1(2): 95-98
54. Bekhechi, C., B.F. Atik, D. Consiglio, A. Bighelli and F. Tomi. 2001. Chemical variability of the essential oil of *Juniperus phoenicea* var. *turbinata* from Algeria. *Biochemical System and Ecology*, 29(2): 179-188.
55. Cavaleiro, C., Rezzi, S., Salgueiro, L., Bighelli, A and Casanova, J. (2001). Comparisons of the leaf essential oils of *Juniperus* Infraspecific chemical variability of the leaf essential phoenicea, J. phoenicea subsp eu-mediterranea oil of *Juniperus phoenicea* var.turbinata from Lebr. and Thiv and J. phoenicea var. turbinata Portugal. *Biochem. Syst. Ecol.*, 29: 1175-1183.
56. Rezzi, S., C. Cavaleiro, A. Bighelli, L. Salgueiro, A.P. da Cunha and J. Casanova, 2011. Intraspecific is still required to analyse the essential oils of this species chemical variability of the leaf essential oil of in the studied region. *Juniperus phoenicea* subsp. *turbinata* from Corsica. *Journal of Food Science*. 76(2): C224-30.
57. Fouad, B., R. Abderrahmane, A. Youssef, H. Rajaand MA El Fels, 2012. Chemical composition and antibacterial activity of the essential oil of Moroccan *Juniperus phoenicea*. *Food Research International* 45(1): 313-319.
58. El-Sawi, S.A., H.M. Motawae and M.A. Amal, (2007). Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicea* grown in Egypt. *African Journal of Traditional Complementary Alternative Medicines*, 4: 417-426.
59. Dob, T., T. Berramdane and C. Chelgoum. (2005). Chemical composition of essential oil of *Pinus halepensis* Miller growing in Algeria C. R. *Chimie* 8(11-12): 1939-1945.

60. El-Zwaam, S.M., 1995. Al-Jabel Al-Akhdar study of natural geography. Garyounis University Publications. Libyan National Library, Benghazi, pp: 139.
61. Alagbe John Olujimi, Ramalan Sadiq Muhammad., Shittu Muritala Daniel and Olagoke Olayemi Christiana (2022). Effect of *Trichilia monadelpha* stem bark extract on the fatty acid composition of rabbit's thigh meat. *Journal of Environmental Issues and Climate Change* 1(1): 63-71.
62. Agubosi, O.C.P., Alexander, James and Alagbe, J.O. (2022). Influence of dietary inclusion of Sunflower (*Helianthus annus*) oil on growth performance and oxidative status of broiler chicks. *Central Asian Journal of Medical and Natural Sciences* 2(7): 187-195.
63. Agubosi, O.C.P., Soliu, M.B and Alagbe, J.O. (2022). Effect of dietary inclusion levels of *Moringa oleifera* oil on the growth performance and nutrient retention of broiler starter chicks. *Central Asian Journal of Theoretical and Applied Sciences* 3(3): 30-39.
64. Lin, J., Opoku, A.R., Geheeb-Keller, M., Hutchings, A.D., Terblanche, S.E., Jager, A.K., van Staden, J. (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology* 68, 267–274.
65. Moreno, L., Bello, R., Primo-Yufera, E., Esplugues, J. (1998b). In vitro studies of methanol and dichlorometanol extracts of *J. oxycedrus* L. *Phytotherapy Research* 11, 309–311.
66. Zgoda, J.R., Porter, J.R. (2001). A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology* 39 (3), 221–225.
67. Alipour, F., Hassanabadi, A., Golian, A and Nassiri, M.H. (2015). Effect of plant extracts derived from thyme on male broiler performance. *Poultry Science*, 94(11): 2630-2634.
68. Oloruntola, O.D., Ayodele, S.O., Agbede, J.O., Oloruntola, D.A., Ogunsipe, M.H and Omoniyi, I.S. (2016). Effect of *Alchornea cordifolia* meal and enzyme supplementation on growth, haematological, immunostimulatory and serum biochemical response of rabbits. *Asian Journal of Biological and Life Sciences*, 5(2): 190-195.
69. Oloruntola, O.D., Ayodele, S.O., Agbede, J.O., Oloruntola, D.A. (2018a). Neem, pawpaw and bamboo leaf meal dietary supplementation in broiler chickens. Effect on performance and health status. *Journal of Food Biochemistry*, e12723. <https://doi.org/10.1111/jfbc.12723>.
70. Sieniawska, M. Swatko-Ossor, R. Sawicki, K. Skalicka-Wozniak, G. Ginalska. (2017). Natural terpenes influence the activity of antibiotics against isolated *Mycobacterium tuberculosis*, *Med. Princ. Pract.* 26 (2017) 108–112, doi:[http:// dx.doi.org/10.1159/000454680](http://dx.doi.org/10.1159/000454680).
71. Marina, R. Mihailo. (2011). Chemical composition and antifungal activities of essential oils from leaves, calyx and corolla of *Salvia brachyodon* Vandas, J. *Essent. Oil Res.* 17 (2011) 22
72. Mughal, T.Z. Khan, M.A. Nasir. (1996). Antifungal activity of some plant extracts, *Pak. J. Phytopath.* 8 (1996) 46–48.
73. Schmidt, J.A. Noletto, B. Vogler, W.N. Setzer, Abaco bush medicine: chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas, *J. Herbs Spices Med. Plants* 12 (2006) 43–65, doi: http://dx.doi.org/10.1300/J044v12n03_04.
74. Nychas, G. (1995). Natural antimicrobial from plants, in: G.W. Gould (Ed.), *New Methods of Food Preservations*, Blackie Academic and Professional, Glasgow, UK, 1995, pp. 58–89.
75. Abdel Rasoul, G.I. Marei, S.A. Abdelgaleil. (2012). Evaluation of antibacterial properties and biochemical effects of monoterpenes on plant pathogenic bacteria, *Afr. J. Microbiol. Res.* 6 (2012) 3667–3672, doi: <http://dx.doi.org/10.5897/AJMR12.118>.

76. Duran, M. Duran, M.B. de Jesus, A.B. Seabra, W.J. Favaro, G. Nakazato. (2015). Silver nanoparticles: a new view on mechanistic aspects on antimicrobial activity, *Nanomed. Nanotechnol. Biol. Med.* 12:(2015) 789–799.
77. International Poultry Production (2001). Full natural phytogetic support for intestinal resilience in poultry. *International Poultry Production Magazine*, 30(4): 25-27.