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Gas Chromatography and Mass Spectroscopy of Juniperus Phoenice Stem Bark Extract and its Influence on the Haemato-Biochemical Values of Growing Rabbits

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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.***

Keywords: ***Juniperus Phoenice, Rabbits, Phytochemicals, Blood, Gas Chromatography, Mass Spectroscopy.***

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1. 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-inflammtory, cytotoxic, hepato-protective, antimutagenic, antiplasmodial, anti-proliferative, antifungal, antiviral, antihelminthic, angelsics, 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-cayrophyllene, 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, Staphyllococcus spp, Klebsiella spp, Micrococcus luteus, Escherichia coli, Citrobacter freundii, Bacillus substilis, 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.

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2. MATERIALS AND METHODS

Experimental site, plant material and extraction procedure

The experiment was carried out at Sumitra Research Institute Gujarat, India (23º 13'N 72º41'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**ºC** 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 m, speed range (20-280 rpm), stroke (150 mm) and temperature (RT – 180 **ºC**). The extract was stored in a white labelled sterile container and kept in the refrigerator at 4 **ºC** 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 **ºC**, pressure range (100 psi ± 0.001 psi), split mode (split/splitless, max split ratio: 1000:1) and column oven working temperature (+4 ºC˜ 450 **ºC**) while MS specifications; EI source ionization energy (5 eV – 250 eV), mass range (1.5 – 1000 amu), ion source temperature (100 -300 **ºC**), stability (± 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 uL 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 × 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 × 70 cm × 35 cm) (Length × width × 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

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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^{TM} 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 spectromer (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

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**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 Ash		162.00 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 _g; 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

3. 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 immunostumilatory 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).

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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-cayrophyllene	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	$C_7H_{14}O_6$	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

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3-methoxy-p-cymene	0.004	$C_{18}H_{21}NO_3$	299.4
4-methyl-2,3-hexadien -1-ol	0.39	$C_7H_{12}O$	112.17
1,2-Cyclopentanedione	0.04	$C_5H_6O_2$	98.10
3-Allyl-6-methoxyphenol	0.003	$C_{10}H_{12}O_2$	164.201
2-Methyl -4-vinylphenol	0.02	$C_9H_{10}O$	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 ($\times10^6$/uL), 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 ($\times10^3$/uL), 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).

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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 secretes anti-toxins and are associated with allergies while basophils releases 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 ($\times10^{6}$/uL)	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 ($\times10^{3}$/uL)	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, α1-globulin, α2-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 α1-globulin, α2-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

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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
α1-globulin (g/dL)	1.78^{c}	3.03^{b}	4.15^{a}	4.33^{a}	0.40
α2-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

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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)

4. 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.

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