Ameliorative Role of Some Herbal Powders and Vitamins in Enhancing the Effectiveness of Antibacterial Agents and Performance of Broiler Chicken's

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Abstract

This study evaluated a polyherbal mixture (PHM) and trace elements as alternatives to antibiotics in poultry production, focusing on performance, carcass composition, lipid profiles, and intestinal morphology under heat stress conditions. The experiment lasted 40 days and used a 2 x 3 factorial design, with trace elements at 0 and 5 g/kg and PHM at 0, 25, and 50 g/kg. Adding varying levels of PHM to the diet significantly increased the relative weight of the Bursa of Fabricius at 5% ($P<0.05$). However, it did not affect other organs, small intestine length, ceca, average weight gain (AWG), or feed intake (AFI) ($P>0.05$). PHM-treated groups also showed lower mortality rates in heat-stressed chickens. Additionally, the inclusion of PHM and trace elements did not significantly alter liver enzyme levels, glucose, uric acid, or lipid profiles compared to the control group ($P>0.05$). However, the group receiving 5% trace elements experienced a notable reduction in triglyceride and very low-density lipoprotein levels ($P<0.05$). Salmonella sp. and E. coli O157:H7 were found in the control and trace element groups but not in the cecal digesta of the PHM groups. The PHM extract demonstrated significant antibacterial effects against these strains and E. coli ATCC at 20 mg compared to other antibiotics. In conclusion, PHM contains bioactive components with antibacterial properties against E. coli and Salmonella species. It reduces these pathogens in the cecal content of heat-stressed chickens, improves feed intake, and lowers mortality rates. Thus, PHM is an effective antibacterial agent for chickens, helping to reduce microbial resistance.

Keywords: Antimicrobial Activity.; Bacteria; Heat Stress; Polyherbal.

Introduction

The poultry industry has evolved into a fast-growing sector worldwide. The increasing global population has heightened the demand for poultry products, requiring rigorous compliance with quality control measures and food safety standards [1]. Antibiotics are routinely incorporated into poultry feed at subtherapeutic doses for

extended durations to enhance growth performance and prevent digestive tract infections in poultry farming [2, 3]. The administration of antibiotics in poultry farming serves valid therapeutic purposes; however, excessive and inappropriate use has raised significant concerns [4]. The improper application of these substances has contributed to the emergence and spread of antimicrobial resistance, posing a significant threat to public health. The increasing prevalence of multidrug-resistant bacteria has resulted in severe infections affecting both humans and animals [5-10].

Also, heat stress adversely affects broiler productivity by diminishing feed consumption, growth rate, and feed conversion efficiency, resulting in suboptimal development and production performance [1]. Additionally, it modifies the intestinal microbial composition, increasing the proliferation of harmful bacteria while reducing populations of beneficial microorganisms, thereby impairing intestinal integrity and immunological responses; such microbial imbalance heightens broilers' vulnerability to infectious diseases and overall health deterioration [11]. The rapid escalation of antimicrobial resistance in pathogenic bacteria underscores the urgent need to explore viable, eco-friendly antibiotic alternatives. These substitutes can be utilized in animal husbandry to sustain production efficiency while combating infections caused by prevalent gastrointestinal pathogens in poultry, such as *Escherichia* and *Salmonella* [12-14]. Many medicinal plants have been traditionally used worldwide for their therapeutic properties [15]. These plants contain various bioactive compounds, such as polyphenols, terpenes, and phytosterols, contributing to their medicinal effects [16]. Herbal immunomodulation has gained attention as an effective traditional treatment, providing preventive and therapeutic benefits for various ailments, including bacterial infections [17].

Natural herbs and plant-derived compounds are widely advocated for their extensive therapeutic benefits. Polyherbal formulations offer a safer and more economical option for preventing and managing immunosuppressive bacterial infections and related diseases. This research aimed to determine the PHM extract's antibacterial properties and evaluate the most effective bactericidal effect using in vitro and in vivo in poultry production. Moreover, the evaluation focused on various parameters, including performance, carcass composition, blood profile, serum biochemical traits, liver enzymes, mortality rate, and intestinal morphology following the supplementation of PHM to the broilers' diet.

Materials and Methods

Powder preparation and analysis

A local company supplied the herbals, including pomegranate peel, olive pomace, black seed, garlic, and rosemary. The herbals were then processed into a fine powder using a laboratory mill, and the Polyherbal Mixture (PHM) was in equal ratios for each herb. Standard laboratory protocols are employed to determine the contents of crude protein, ether extract (fat), crude fiber, and ash to analyze its nutritional composition. These analyses are conducted following the methods outlined by the [18].

Extraction of phenolic compounds

The ethanolic extraction of the PHM was performed by macerating 10 g of air-dried HM powder in 100 mL of 99.8% ethanol, followed by shaking at 85 rpm and 25°C for 6 hours. The resulting extract was filtered through Whatman No. 1 filter paper using a Buchner funnel, and the filtrate was concentrated to dryness under reduced pressure using a rotatory vacuum evaporator. The dried extract was stored in the dark at 4°C until GC-MS analysis. The GC-MS analysis was conducted using an Agilent 7890A Gas Chromatograph coupled with an electron impact quadrupole MD 800 mass spectrometer and a 19,095–400 fused silica column (30 m length, 0.25 mm inner diameter, and 0.25 µm stationary phase thickness). The column temperature was initially set at 35°C for 2.50 minutes and then gradually increased to 280°C over 20 minutes at increments of 7°C. The volatile oil components were identified based on their GC Kovats retention indices.

Inoculum's preparation

Each bacterial strain was subcultured in Mueller-Hilton agar slants for 24 hours at 35 ℃. The bacterial growth was obtained using 5 milliliters of sterile saline water. Its absorbance was then adjusted to 580 um and diluted to attain a viable cell count of 107 CFU/ml using a spectrophotometer.

Antibacterial assay

The disk diffusion method assesses the PHM extract's antibacterial properties. In order to obtain a final concentration of 20 mg/disc, the PHM extract residues (100 mg) were re-dissolved in 2.5 ml of ethanol, sterilized through a Millipore filter (0.22 mm), and then loaded over sterile filter paper discs (8 mm in diameter). The discs were then inoculated with bacterial suspension (100 ml of medium/1 ml of 107 CFU). Mueller-Hilton agar plates were topped with sterile filter paper discs with a 10 mg/ml PHM extract concentration. Filter paper discs that are filled with (Gentamicin 10 mcg, Tetracycline 30 mcg, Neomycin 30 mcg, Amoxicillin 25 mcg, Colistin 10 mcg, Streptomycin 10 mcg, Levofloxacin 5 mcg, Erythromycin15 mcg, Bactrime 30 mcg, and Ciprofloxacin 30 mcg) were used as positive control. The plates were kept in the fridge at 5℃ for 2 h. to permit PHM extract diffusion, then incubated at 35 ℃ for 24 h. The presence of inhibition zones was measured by Vernier caliper, recorded, and considered as an indication of antibacterial activity.

Bird, Housing and Feeding

The experiment was carried out at the University of Raparin, Kurdistan Region-Iraq. The University of Raparine Animal Care and Use Committee assesses and approves all animal care and use methods per international principles (FASS, 2010). The experiment spanned 40 days, using a 2 x 3 factorial design, with the first factor a mixture of named Trace elements (TE) at 0 and 5 g/kg, which is composed of selenium 60mg, zink 500mg, vitamin C 500 IU, and vitamin E 500 IU and the second-factor PHM at 0, 25, and 50 g/kg. 300 one-day-old ross chicks were randomly allocated to

six treatments in five replicated 50 chicks for each. The experiment was conducted for 40 days, during which the subjects had unrestricted access to feed and water. The diet was divided into three phases: starter (1–11 days), grower (12–25 days), and finisher (26–40 days), all in the form of meal pellets.

Table (1): Diet rations for broiler chickens from 1-11 days

Ingredients	Control		2.5% Polyherbal Mixture		5% Polyherbal Mixture	
	No TE	With TE	No TE	With TE	No TE	With TE
Corn grain	55.698	54.760	53.137	52.199	50.577	49.639
Soybean Meal 46%	37.214	37.373	37.161	37.320	37.108	37.267
Sunflower oil	1.761	2.038	1.857	2.133	1.952	2.229
Dicalcium Phosphate	1.304	1.307	1.318	1.321	1.333	1.336
Calcium Carbonate	1.036	1.034	1.029	1.027	1.023	1.020
Common Salt	0.132	0.133	0.134	0.134	0.135	0.135
Concentrate[1]	2.500	2.500	2.500	2.500	2.500	2.500
Methionine	0.355	0.000	0.364	0.365	0.373	0.374
Polyherbal Mixture	0.000	0.000	2.500	2.500	5.000	5.000
Trace Elements	0.000	0.500	0.000	0.500	0.000	0.500
	Chemical composition					
M. Energy kcal/kg	2.975	2.975	2.975	2.975	2.975	2.975
Crude Protein%	23.000	23.000	23.000	23.000	23.000	23.000
Linoleic acid%	2.272	2.403	2.265	2.395	2.257	2.387
Crude Fiber%	3.379	3.359	3.810	3.789	4.240	4.220
Calcium%	1.031	1.031	1.031	1.031	1.031	1.031
Available Phosphate%	0.500	0.500	0.500	0.500	0.500	0.500
Potassium%	0.913	0.914	0.905	0.905	0.896	0.896
Sodium%	0.180	0.180	0.180	0.180	0.180	0.180
Lysine%	1.274	1.276	1.266	1.269	1.259	1.261
Methionine%	0.717	0.718	0.722	0.722	0.727	0.727
TSAA%	1.000	1.000	1.000	1.000	1.000	1.000
Threonine%	0.853	0.853	0.844	0.844	0.836	0.386

Abbreviations: TE; Trace elements, M. Energy; Metabolizable Energy, TSAA; Total sulfuric amino acids.
[1]. Provided per kg of diet: Protein, 18%; CaCO3, 19%; Monocalcium phosphate, 7%; NaCl, 4.5%; Methionine, 2%; Lysine, 4%; Threonine, 1%; Choline chloride, 1%; Iron (sulphate), 2800mg; Zinc (oxide), 2480mg; Manganese (oxide), 3600mg; Copper (sulphate), 240mg; Iodine (IK), 60mg; Selenium (SeNa), 14mg; Cobalt (sulphate), 14mg;

Magnesium (oxide), 800mg; Antioxidant (BHT), 32mg; Vitamin A, 420000 IU; Vitamin D3, 80000 IU; Vitamin E, 800mg; Vitamin C, 40mg; Vitamin K3, 80mg; Vitamin B1, 80mg; Vitamin B2, 200mg; Vitamin B6, 80mg; Vitamin B12, 0.6 mcg; Biotin, 10.4mg; Folic acid, 20mg; Nicotinic acid, 400mg; Pantothenic acid, 160mg.

The chicks were kept at a temperature above 35°C for the first three days and provided with 24 hours of light. After this initial period, the temperature was adjusted to 35°C ± 2°C to simulate chronic heat stress. The lighting schedule was 23 hours of light followed by 1 hour of darkness, continuing until they reached 40 days of age.

Table (2): Diet rations for broiler chickens from 12-25 days

Ingredients	Control		2.5% Polyherbal Mixture		5% Polyherbal Mixture	
	No TE	With TE	No TE	With TE	No TE	With TE
Corn grain	59.031	58.231	56.179	55.332	53.292	52.363
Soybean Meal 46%	33.602	33.697	33.836	33.905	34.104	34.254
Sunflower oil	2.647	2.891	2.753	3.027	2.860	3.137
Dicalcium Phosphate	0.910	0.913	0.922	0.926	0.934	0.937
Calcium Carbonate	0.869	0.867	0.861	0.859	0.854	0.852
Common Salt	0.132	0.081	0.134	0.134	0.135	0.136
Concentrate[1]	2.500	2.500	2.500	2.500	2.500	2.500
Methionine	0.308	0.320	0.315	0.317	0.321	0.322
Polyherbal Mixture	0.000	0.000	2.500	2.500	5.000	5.000
Trace Elements	0.000	0.500	0.000	0.500	0.000	0.500
	Chemical composition					
M. Energy kcal/kg	3.050	3.050	3.050	3.050	3.050	3.050
Crude Protein%	21.514	21.500	21.626	21.591	21.751	21.748
Linoleic acid%	2.783	2.900	2.775	2.906	2.768	2.898
Crude Fiber%	3.310	3.291	3.745	3.723	4.181	4.160
Calcium%	0.870	0.870	0.870	0.871	0.871	0.871
Available Phosphate%	0.420	0.420	0.420	0.420	0.420	0.420
Potassium%	0.851	0.850	0.847	0.846	0.843	0.844
Sodium%	0.180	0.160	0.180	0.180	0.180	0.180
Lysine%	1.180	1.181	1.180	1.180	1.181	1.183
Methionine%	0.655	0.665	0.658	0.659	0.661	0.662
TSAA%	0.920	0.930	0.920	0.920	0.920	0.920
Threonine%	0.798	0.797	0.794	0.792	0.790	0.790

Abbreviations: TE; Trace elements, M. Energy; Metabolizable Energy, TSAA; Total sulfuric amino acids.
[1]. Provided per kg of diet: Protein, 18%; CaCO3, 19%; Monocalcium phosphate, 7%; NaCl, 4.5%; Methionine, 2%; Lysine, 4%; Threonine, 1%; Choline chloride, 1%; Iron (sulphate), 2800mg; Zinc (oxide), 2480mg; Manganese (oxide), 3600mg; Copper (sulphate), 240mg; Iodine (IK), 60mg; Selenium (SeNa), 14mg; Cobalt (sulphate), 14mg; Magnesium (oxide), 800mg; Antioxidant (BHT), 32mg; Vitamin A, 420000 IU; Vitamin D3, 80000 IU; Vitamin E, 800mg; Vitamin C, 40mg; Vitamin K3, 80mg; Vitamin B1, 80mg; Vitamin B2, 200mg; Vitamin

B6, 80mg; Vitamin B12, 0.6 mcg; Biotin, 10.4mg; Folic acid, 20mg; Nicotinic acid, 400mg; Pantothenic acid, 160mg.

Growth performance

The initial weights of the birds were recorded individually, and the average body weight for each treatment group was determined at 11, 25, and 40 days. Weight gain at each interval was calculated as the difference between the final and initial body weights. The average feed intake per bird in each replicate was estimated by subtracting the residual feed from the total feed provided and dividing by the number of birds in the replicate. The feed conversion ratio (FCR) was calculated as feed divided by weight gain.

Table (3): Diet rations for broiler chickens from 26-40 days

Ingredients	Control		2.5% Poly-herbal Mixture		5% Polyherbal Mixture	
	No TE	With TE	No TE	With TE	No TE	With TE
Corn grain	62.605	60.000	60.000	59.034	57.029	56.182
Soybean Meal 46%	29.867	30.308	29.800	30.043	30.208	30.277
Sunflower oil	3.193	3.962	3.299	3.568	3.401	3.674
Dicalcium Phosphate	0.624	1.265	0.661	0.641	0.649	0.653
Calcium Carbonate	0.745	0.995	0.747	0.736	0.731	0.728
Common Salt	0.183	0.183	0.185	0.185	0.186	0.186
Concentrate[1]	2.500	2.500	2.500	2.500	2.500	2.500
Methionine	0.283	0.286	0.309	0.293	0.297	0.299
Polyherbal Mixture	0.000	0.000	2.500	2.500	5.000	5.000
Trace Elements	0.000	0.500	0.000	0.500	0.000	0.500
	Chemical composition					
M. Energy kcal/kg	3.100	3.100	3.100	3.100	3.100	3.100
Crude Protein%	20.000	20.000	20.000	20.028	20.175	20.140
Linoleic acid%	3.125	3.471	3.122	3.248	3.110	3.240
Crude Fiber%	3.242	3.185	3.670	3.653	4.110	4.088
Calcium%	0.750	0.987	0.758	0.750	0.750	0.751
Available Phosphate%	0.360	0.478	0.364	0.360	0.360	0.360
Potassium%	0.786	0.788	0.777	0.779	0.776	0.775
Sodium%	0.200	0.200	0.200	0.200	0.200	0.200
Lysine%	1.083	1.090	1.075	1.080	1.080	1.080
Methionine%	0.612	0.614	0.634	0.617	0.620	0.621
TSAA%	0.860	0.860	0.877	0.860	0.860	0.860
Threonine%	0.741	0.742	0.732	0.734	0.731	0.730

Abbreviations: TE; Trace elements, M. Energy; Metabolizable Energy, TSAA; Total sulfuric amino acids.
[1]. Provided per kg of diet: Protein, 18%; CaCO3, 19%; Monocalcium phosphate, 7%; NaCl, 4.5%; Methionine, 2%; Lysine, 4%; Threonine, 1%; Choline chloride, 1%; Iron (sulphate), 2800mg; Zinc (oxide), 2480mg; Manganese (oxide), 3600mg; Copper (sulphate), 240mg; Iodine (IK), 60mg; Selenium (SeNa), 14mg; Cobalt (sulphate), 14mg;

Magnesium (oxide), 800mg; Antioxidant (BHT), 32mg; Vitamin A, 420000 IU; Vitamin D3, 80000 IU; Vitamin E, 800mg; Vitamin C, 40mg; Vitamin K3, 80mg; Vitamin B1, 80mg; Vitamin B2, 200mg; Vitamin B6, 80mg; Vitamin B12, 0.6 mcg; Biotin, 10.4mg; Folic acid, 20mg; Nicotinic acid, 400mg; Pantothenic acid, 160mg.

Blood and serum collection

The blood sample was collected from the brachial vein of one hen per pen 40 days before slaughter, centrifuged at 3000 rpm for 15 minutes to separate the serum, and stored at -20°C until analysis. Serum liver enzymes, including alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST), Uric acid, total protein, and lipid profile levels were measured calorimetrically using professional kits Biolabo S.A.S, Les Hautes Rives, 02160 Maizy, France [19].

Cecal Microbiota Diversity

To detect *Salmonella sp.* and *Escherichia coli* in the cecum, the Applied Biosystems™ 7500 Fast Real-Time PCR System was employed, along with the Applied Biosystems™ RapidFinder™ Express Software v2.0.

Carcass Traits

On day 40, birds from each treatment group were selected for carcass evaluation based on their average body weight specific to that treatment. These birds were slaughtered, de-feathered, and eviscerated, after which their carcasses were weighed to determine the dressing percentage. The weights of the dressed carcass, viscera, intestine, liver, heart, and spleen were recorded and expressed as a percentage of the live body weight.

Histopathological Examination of the Small Intestine

The intestinal specimens were preserved in 10% neutral buffered formalin for fixation until histological examination. The samples underwent dehydration using increasing ethanol concentrations (75–100%), were treated with xylol I and II, embedded in paraffin, and sectioned into 4 mm lengths and widths using a microtome (Leica RM 2155, England). The sections were stained with hematoxylin and eosin following Suvarna et al.'s protocol. Villus height, crypt depth, villus height-to-crypt depth ratio, and villus surface area were measured at 40x magnification using a compound light microscope equipped with an AmScope digital camera and analyzed with AmScope R software. For further analysis, the average values were calculated from measurements of 10 villi per sample [20].

Analytical Statistics

All collected data were analyzed using the General Linear Model procedures from the SAS Institute (2001) [21]. The individual bird was used as the experimental unit for all parameters except for performance and mortality, where the cage was considered the experimental unit. When the model indicated significant effects, mean differences were evaluated using Tukey's test, with a significance level set at $P < 0.05$.

Results and Discussion

Polyherbal Mixture Chemical Composition and Bioactive Compounds

Table 4 shows the chemical composition of PHM, and **Table 5 and Figure 1** show the various bioactive compounds present in the PHM extract, each with distinct biological activities. Seventeen bioactive compounds were identified according to GC-MS analysis, most demonstrating antimicrobial and antibacterial effects. Linoleic acid is the predominant compound, making up approximately 42% of the total bioactive content. After linolic acid, the most compounds in the mixture include oleic acid, palmitic acid, elaidic acid, and stearic acid.

Table (4): Chemical composition of polyherbal mixture on a dry matter basis

Ingredients	Chemical composition %							
	DM	Moisture	CP	EE	Ash	CF	NFE	ME (kCal/kg)
polyherbal mixture	92.33	7.67	9.21	10	4.19	20.4	48.53	2871

Abbreviations: DM; Dry matter, CP; Crude protein, EE; Ether extract, CF; Crude fiber, NFE; Nitrogen free extract, ME; Metabolizable energy.

Table (5): Bioactive compound present in the Polyherbal mixture extract indicated by GC-MS

#	Compounds	Common name	Retention Time	%	Bioactivity	Reference
1	Eucalyptol		7.893	0.12	Antimicrobial	[22]
2	Camphor		10.41	0.08	Antimicrobial	[23]
3	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	Levo verbenone	11.851	0.15	Antibacterial	[24]
4	Beta- Caryophyllene		15.916	0.04	Antibacterial	[25]
5	Carteolol AC		17.757	0.42		
6	Tetradecanoic acid, ethyl ester	Myristic acid	22.045	0.48	Antibacterial	Awa et al., 2012
7	Palmitoleic acid		24.427	0.07	Antibacterial	[26]
8	n-Hexadecanoic acid	Palmitic acid	24.615	7.56	Antibacterial	[27]
9	Hexadecanoic acid, ethyl ester	Palmitic acid	24.897	4.87	Antibacterial	[28]

10	9,12-Octadecadi-enoic acid (Z,Z)-	Linoleic acid	26.262	42.87	Antibacte-rial	[29]
11	11-Octadecenoic acid, methyl ester	Methyl 11-octadecen-oate	26.338	0.04	Antibacte-rial	[30]
12	Linoleic acid ethyl ester		27.156	18.49	Antibacte-rial	[31]
13	(E)-9-Octade-cenoic acid ethyl ester	Elaidic acid	27.227	17.95	Antibacte-rial	[32]
14	Octadecanoic acid, ethyl ester	Stearic acid	27.527	4.74	Antibacte-rial	[33]
15	Linoelaidic acid		29.344	0.26	Antibacte-rial	[34]
16	cis-13,16-Docasa-dienoic acid		29.591	1.21	Antioxi-dant	[35]
17	Phenol		30.438	0.14	Antibacte-rial	[36]
	SUM			100		

Table 6 illustrates the effects of PHM and trace elements on the performance and mortality rate of broiler chickens exposed to chronic heat stress from 1 to 40 days of age. The supplementation of PHM and TE at different levels did not influence ($P>0.05$) ABW during all rearing phases (starter, grower, and finisher). Also, the interaction effect between PHM and trace element supplementation was not recorded ($P>0.05$) on the ABW in chickens exposed to chronic heat stress. In addition, supplementing different levels of PHM did not influence AWG in all rearing phases compared to the control group, except in the grower phase, where supplementation of 2.5% PHM reduced ($P<0.05$) AWG compared to the control group while adding 5% PHM did not significantly reduce AWG in grower phase compared to the control group ($P>0.05$). Adding trace elements did not change AWG in chickens exposed to chronic heat stress compared to the control group in all rearing phases ($P>0.05$). However, the interaction effect between trace elements and PHM on AWG was not recorded in chickens exposed to heat stress($P>0.05$) in all rearing phases except the grower phase; the supplementing 0.5g/kg of trace element and PHM 5% with 0.5g/kg trace elements significantly enhances the AWG compared to all experimental groups. The mortality rate between experimental groups did not change in chickens reared under chronic heat stress conditions ($P>0.05$).

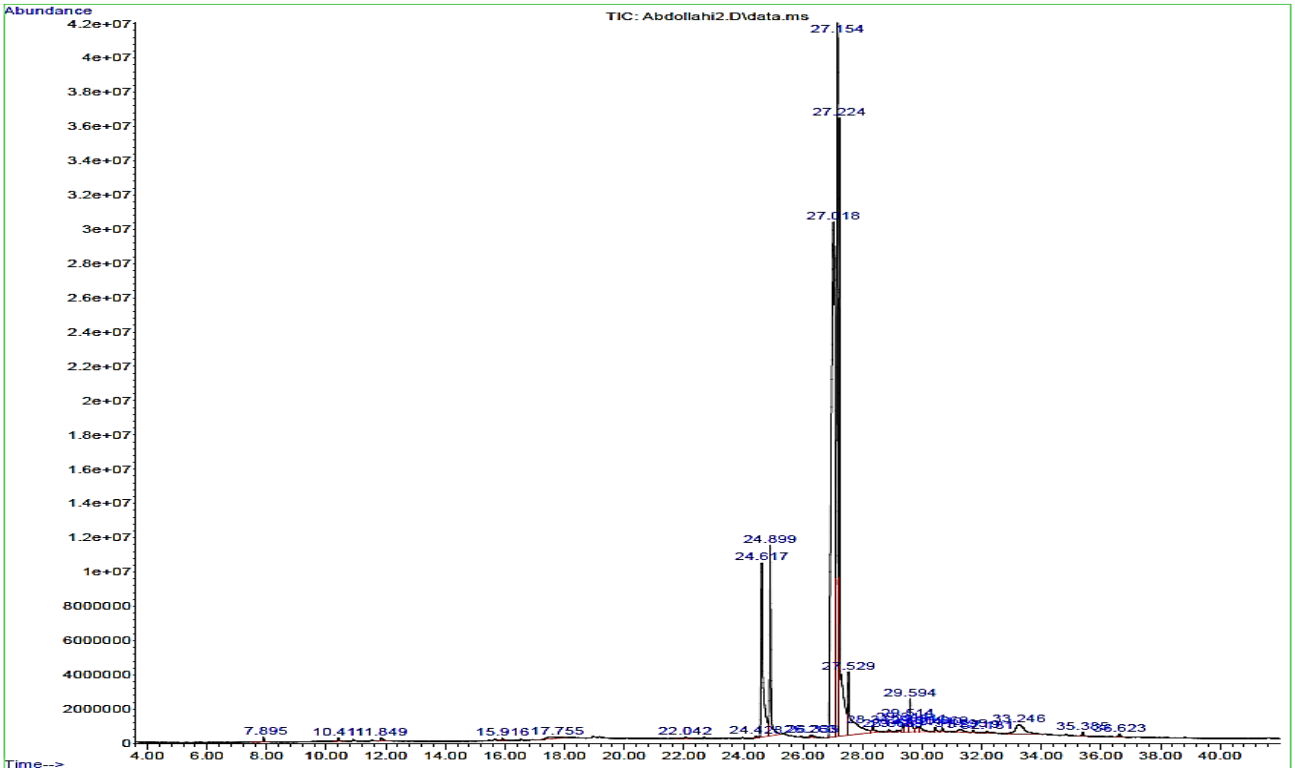

Figure 1: Bioactive compounds detected by GC-MS in polyherbal mixture extract

Table 7 shows the effects of PHM and trace elements on the AFI and FCR in broiler chickens exposed to chronic heat stress from 1 to 40 days of age. Supplementing PHM to the diet of chicks at the starter phase reduced ($P<0.05$) AFI compared to the control at level 5% but did not influence AFI in the grower phase ($P>0.5$). Otherwise, the inclusion of PHM in 2.5 and 5% levels to the diet of broiler chickens exposed to chronic heat stress significantly improved AFI compared to the control group in the finisher phase and 1-40 days of age. On the other hand, adding trace elements to the diet did not influence AFI in both starter and grower phases ($P>0.05$). In contrast, the finisher phase with 1-40 days of age significantly enhances AFI in chickens fed a diet supplemented with 0.5g/kg trace elements ($P<0.05$). In addition, the interaction effect between PHM and trace elements was recorded in the 2.5% PHM and 0.5g/kg trace element experimental group, which recorded the highest AFI among other experimental groups at the finisher phase and all rearing period (1-40 days).

In the starter and grower phases, supplementing the diet of broiler chicks with trace elements or PHM at different levels or combinations did not significantly change FCR compared to the control group. However, In the finisher phase and all rearing period (140 days), supplementing PHM or trace elements to the diet of broiler chickens significantly ($P<0.05$) increased FCR compared to the control group. In addition, the interaction between 2.5% PHM and 0.5g/kg of trace elements in the diet of broiler chickens significantly ($P<0.05$) increased FCR compared to the control group and all other experimental groups.

Table (6): Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on average body weight, average weight gain, and mortality rate in broiler chickens exposed to chronic heat stress from 1 to 40 days of age

Dietary treatments		Average Body Weight (g)			Average Weight Gain (g)				Death %
PHM level	TE level	11 days	25 days	40 days	1-11 day	12-25 day	26-40 day	1-40 day	1-40 day
0%	0 g/kg	284	930	1959	244	646 bc	1029	1919	18
0%	0.5 g/kg	258	979	1891	218	721 a	1029	1851	18
2.5%	0 g/kg	256	932	1762	216	676 bc	830	1722	16
2.5%	0.5 g/kg	259	884	1841	219	626 c	957	1801	14
5%	0 g/kg	253	958	1886	213	704 ab	928	1846	14
5%	0.5 g/kg	257	970	1883	217	714 a	904	1843	20
Polyherbal Mixture level									
0% PHM		271	954	1925	231	683 ab	1029 a	1885	18
2.5% PHM		258	908	1802	218	651 b	893 b	1762	15
5% PHM		255	964	1885	215	709 a	918 ab	1845	17
Trace elements level									
0 g/kg		265	940	1869	225	675	929	1829	16
0.5 g/kg		258	945	1872	218	687	963	1832	17.3
Source of variation		----------Probability----------							
PHM		0.1307	0.0596	0.2526	0.1307	0.0452	0.0332	0.2526	0.9285
Trace elements		0.3278	0.8163	0.9638	0.3278	0.5380	0.4212	0.9638	0.8383
PHM X Trace Elements		0.1468	0.1429	0.6122	0.1468	0.0313	0.2940	0.6122	0.8715
Pooled SEM		3.608	10.56	29.57	3.608	10.49	23.16	29.57	2.968

In-vitro Antibacterial assay

Figure 2 displays the antimicrobial effects of PHM ethanolic extracts tested against various Salmonella sp., E. coli 0157H7 and E. coli ATCC. bacteria in vitro. The PHM extract demonstrated effectiveness against Salmonella sp. at a concentration of 20 mg. The inhibition zone for this bacterium was recorded as 39mm, which is more potent than levofloxacin and tetracycline or other synthetic antibiotics ($P<0.05$). Most antibiotics lost their antimicrobial effects against E. coli; the most potent are levofloxacin and ciprofloxacin. However, the PHM extract shows the most significant ($P<0.05$) antimicrobial effect against E. coli compared to all other synthetic antimicrobials.

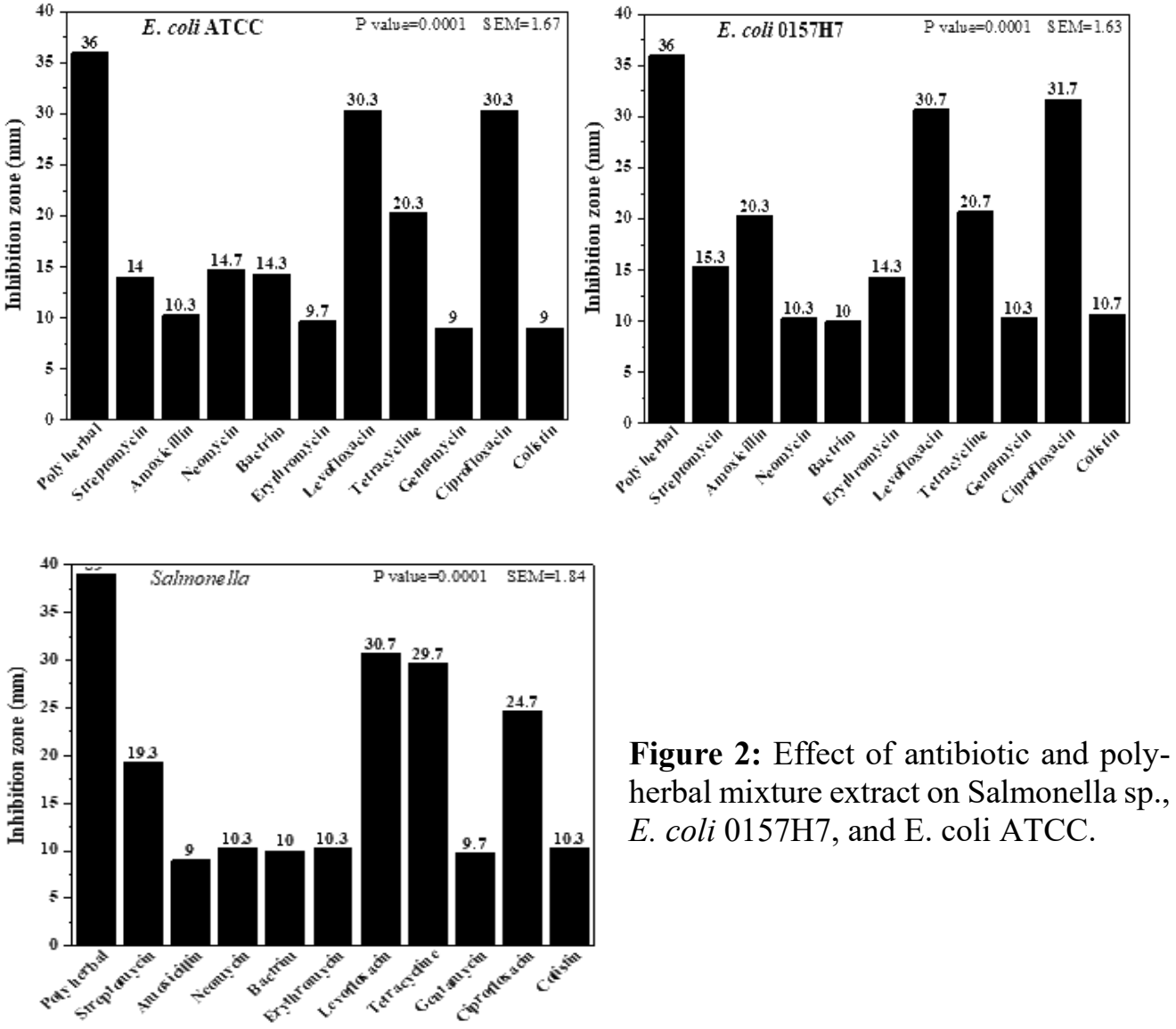

Figure 2: Effect of antibiotic and poly-herbal mixture extract on Salmonella sp., *E. coli* 0157H7, and E. coli ATCC.

Internal Organs Relative Weight and Length

Table 8 presents the impact of PHM and trace elements or a combination of them on internal organs' relative weight under chronic heat stress conditions. Adding different PHM levels into broiler chickens' diet did not significantly change ($P>0.05$) the relative weight of carcass, crop, proventriculus, gizzard, spleen, liver, heart, and

abdominal fat compared to the control group. However, adding 2.5% PHM into the diet significantly reduced the pancreas's relative weight compared to the control and 5% PHM experimental groups, and also, the relative weight of the bursa of Fabricius increased by adding 5% PHM into the diet compared to the control group. In addition, adding trace elements to the diet of the broiler chickens under chronic heat stress conditions did not affect the relative weight of the internal organs (P>0.05). Also, the interaction effect between trace elements and PHM on the relative weight of the internal organs was not shown compared to the control group (P>0.05).

Table 7: Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on average feed intake and feed conversion ratio in broiler chickens exposed to chronic heat stress from 1 to 40 days of age

Dietary treatments		**Average Feed Intake (g)**					**Feed Conversion Ratio (g/g)**			
PHM level	**TE level**	**1-11 day**	**12-25 day**	**26-40 day**	**1-40 day**		**1-11 day**	**12-25 day**	**26-40 day**	**1-40 day**
0%	0 g/kg	284	991	1250 ^{d}	2496 ^{c}		1.172	1.484	1.225 ^{c}	1.306 ^{c}
0%	0.5 g/kg	258	1006	1717 ^{c}	2982 ^{b}		1.127	1398	1.905 ^{b}	1.622 ^{b}
2.5%	0 g/kg	249	940	1817 ^{ab}	3007 ^{b}		1.156	1.393	1.949 ^{ab}	1.632 ^{b}
2.5%	0.5 g/kg	246	934	1961 ^{a}	3141 ^{a}		1.174	1.495	2.062 ^{a}	1.747 ^{a}
5%	0 g/kg	243	976	1774 ^{bc}	2993 ^{b}		1.140	1.388	1.920 ^{b}	1.625 ^{b}
5%	0.5 g/kg	251	965	1768 ^{bc}	2985 ^{b}		1.159	1.352	1.931 ^{ab}	1.626 ^{b}
Polyherbal Mixture level										
0% PHM		270 ^{a}	999	1510 ^{b}	2739 ^{b}		1.149	1.437	1.616 ^{b}	1.481 ^{b}
2.5% PHM		248 ^{ab}	937	1889 ^{a}	3074 ^{a}		1.164	1.444	2.012 ^{a}	1.696 ^{a}
5% PHM		247 ^{b}	971	1770 ^{a}	2989 ^{a}		1.149	1.370	1.925 ^{a}	1.626 ^{a}
Trace elements level										
0 g/kg		257	969	1640 ^{b}	2832 ^{b}		1.155	1.417	1.725 ^{b}	1.529 ^{b}
0.5 g/kg		252	969	1816 ^{a}	3036 ^{a}		1.154	1.415	1.969 ^{a}	1.665 ^{a}
Source of variation		----------Probability----------								

PHM	0.0223	0.1010	0.0001	0.0020		0.8123	0.0678	0.0010	0.0001
Trace elements	0.3610	0.9804	0.0037	0.0079		0.9026	0.8085	0.0055	0.0004
PHM X Trace Elements	0.1953	0.8771	0.0179	0.0245		0.4513	0.0321	0.0108	0.0046
Pooled SEM	4.056	11.25	48.26	49.57		0.0106	0.016	0.062	0.029

Abbreviations: PHM; polyherbal mixture, TE; Trace elements; SEM; sum of standard errors mean

[a-c.] Means with different latter in the same column significantly differ (P<0.05).

Table 9 shows the impact of PHM and trace elements or their combination on intestinal relative weight and length under chronic heat stress conditions. Adding different PHM levels and trace elements into broiler chickens' diet did not significantly change (P>0.05) the relative weight of duodenum, jejunum, ileum, and ceca compared to the control group. Also, adding PHM and trace elements into the diet did not significantly influence the relative length of the duodenum, jejunum, ileum, and ceca compared to the control group (P>0.05). In addition, the interaction effect between trace elements and PHM on the relative weight and length of the internal organs was not shown compared to the control group (P>0.05).

Serum Biochemical Traits

Table 10 shows the impacts of PHM and trace elements or their combination on broiler chickens' serum lipid profile, liver enzymes, glucose, and uric acid levels under heat stress conditions. Supplementing different levels of PHM into the diet did not significantly (P>0.05) affect the lipid profile (total cholesterol, triglyceride, LDL, VLDL, HDL), serum liver enzymes (AST, ALT, and ALP), uric acid, and glucose level compared to the control group. Also, adding trace elements into the diet did not influence serum liver enzyme levels, uric acid, glucose, LDL, HDL, and total cholesterol levels compared to the control group. However, triglyceride and VLDL levels significantly declined compared to the control group by adding 0.5g/kg trace elements into the diet of the broiler chickens (P<0.05). Otherwise, the significant interaction impact between PHM and trace elements in the diet was not recorded on the serum lipid profile, liver, enzymes, uric acid, and glucose level compared to the control group (P>0.05) in chickens reared under chronic heat stress conditions.

Table (8): Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on relative internal organs weight (%) in broiler chickens exposed to chronic heat stress from 1 to 40 days

Dietary treatments		Carcass	Crop	Proventriculus	Gizzard	Spleen	Liver	Pancreas	Bursa of Fabricius	Heart	Abdominal fat
PHM level	TE level										
0%	0 g/kg	62.41	0.353	0.281	0.643	0.062	1.985	0.153	0.036	0.241	0.961
0%	0.5 g/kg	64.33	0.287	0.317	0.640	0.046	1.480	0.135	0.033	0.244	0.747
2.5 %	0 g/kg	64.31	0.287	0.238	0.575	0.058	1.445	0.115	0.042	0.230	0.798
2.5 %	0.5 g/kg	63.99	0.276	0.294	0.649	0.048	1.568	0.131	0.042	0.237	0.739
5%	0 g/kg	64.52	0.240	0.278	0.628	0.051	1.517	0.140	0.047	0.233	0.803
5%	0.5 g/kg	62.94	0.310	0.244	0.618	0.061	1.364	0.151	0.047	0.218	0.788
Polyherbal Mixture level											
0% PHM		63.37	0.324	0.299	0.641	0.054	1.705	0.143 ^{a}	0.034 ^{b}	0.243	0.866
2.5% PHM		64.15	0.281	0.266	0.608	0.053	1.507	0.123 ^{b}	0.042 ^{ab}	0.233	0.768
5% PHM		63.73	0.275	0.261	0.623	0.056	1.441	0.146 ^{a}	0.047 ^{a}	0.226	0.795
Trace elements level											
0 g/kg		63.84	0.293	0.266	0.613	0.057	1.625	0.135	0.041	0.234	0.854
0.5 g/kg		63.71	0.291	0.285	0.635	0.052	1.471	0.139	0.041	0.233	0.759

Source of variation	----------Probability----------									
PHM	0.8031	0.2568	0.2722	0.7073	0.9071	0.1075	0.0091	0.0375	0.5076	0.6023
Trace elements	0.9952	0.9146	0.3474	0.4763	0.2719	0.1201	0.6006	0.8883	0.8625	0.1805
PHM X Trace Elements	0.3482	0.0748	0.1805	0.4134	0.1258	0.0931	0.0720	0.9122	0.6731	0.4953
Pooled SEM	0.451	0.012	0.010	0.013	0.003	0.061	0.004	0.002	0.005	0.034

Abbreviations: PHM; polyherbal mixture, TE; Trace elements; SEM; sum of standard errors mean

[a-c.] Means with different latter in the same column significantly differ (P<0.05).

Intestinal histomorphology

The effects of the PHM and trace elements on the intestinal histomorphology of broilers are shown in **Table 11**. The supplementation of PHM and trace elements in combination with the broilers' diet did not significantly affect the intestinal villus length, crypt depth, villus length to crypt depth ratio, and villus surface area compared to the control group (P>0.05). Also, adding PHM into the diet did not affect the intestinal morphology in the duodenum, jejunum, and ileum compared to the control group (P>0.05) except in the villi surface area in the duodenum, which declined with adding 5%PHM into the diet of the chickens. Also, adding trace elements into the diet did not influence the villi and crypts in the jejunum and ileum compared to the control group. However, adding trace elements into the diet significantly reduced villi length, crypt depth, and villi surface area compared to the control group.

Table (9): Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on relative intestinal weight (%) and length (%) in broiler chickens exposed to chronic heat stress from 1 to 40 days

Dietary treatments		Duodenum		Jejunum		Ileum		Ceca	
PHM level	TE level	Weight	Length	Weight	Length	Weight	Length	Weight	Length
0%	0 g/kg	0.494	1.724	1.324	3.958	0.983	3.851	0.489	1.084
0%	0.5 g/kg	0.499	1.595	1.285	3.589	1.020	3.465	0.371	0.850
2.5%	0 g/kg	0.531	1.695	1.391	3.691	1.055	3.663	0.508	0.957

2.5%	0.5 g/kg	0.404	1.744	1.370	3.672	1.007	3.648	0.343	1.000
5%	0 g/kg	0.460	1.642	1.333	3.757	1.086	3.989	0.459	1.019
5%	0.5 g/kg	0.533	1.875	1.228	4.488	1.117	4.074	0.525	1.132
Polyherbal Mixture level									
0% PHM		0.497	1.660	1.304	3.774	1.003	3.658	0.430	0.967
2.5% PHM		0.475	1.726	1.382	3.682	1.031	3.656	0.417	0.978
5% PHM		0.497	1.771	1.286	4.123	1.101	4.031	0.492	1.076
Trace elements level									
0 g/kg		0.495	1.690	1.349	3.802	1.045	3.834	0.484	1.020
0.5 g/kg		0.484	1.738	1.294	3.917	1.048	3.723	0.413	0.994
Source of variation		----------Probability----------							
PHM		0.8136	0.5204	0.7142	0.2713	0.7958	0.4686	0.4672	0.3695
Trace elements		0.7054	0.4953	0.5949	0.6222	0.9572	0.7119	0.1504	0.7067
PHM X Trace Elements		0.1579	0.1340	0.9390	0.1566	0.9513	0.7753	0.1450	0.1088
Pooled SEM		0.021	0.037	0.047	0.118	0.056	0.134	0.025	0.035

Abbreviations: PHM; polyherbal mixture, TE; Trace elements; SEM; sum of standard errors mean

[a-c.] Means with different latter in the same column significantly differ (P<0.05).

Cecal Microbiota Diversity

Figures 3 and 4 show a section of a qPCR (quantitative Polymerase Chain Reaction). They include amplification curves for two assays: one targeting *E. coli O157:H7* and another targeting *Salmonella spp*. In the first graph (orange curve), fluorescence increases sharply after approximately 23–25 cycles, indicating the presence of *E. coli* DNA in control and trace elements 0.5g/kg experimental groups, while in other experimental groups containing PHM, the DNA of *E. coli* was not recorded. The second graph (blue curve) shows a similar amplification trend for *Salmonella spp*., with an apparent rise in fluorescence after about 22–24 cycles in the control group. In contrast, it was not recorded in other experimental groups. Both pathogens were detected in the control except for different levels of the PHM.

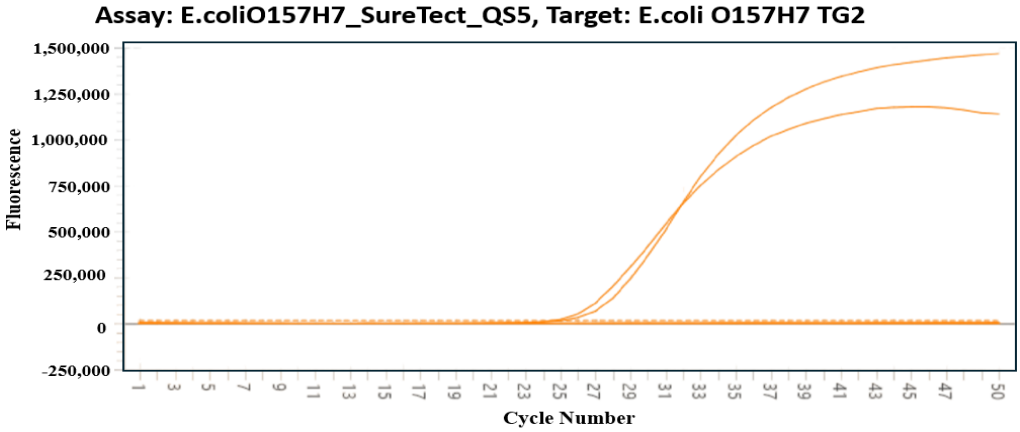

Figure 3: *E. coli* 0157H7 detection in cecal digesta by qPCR

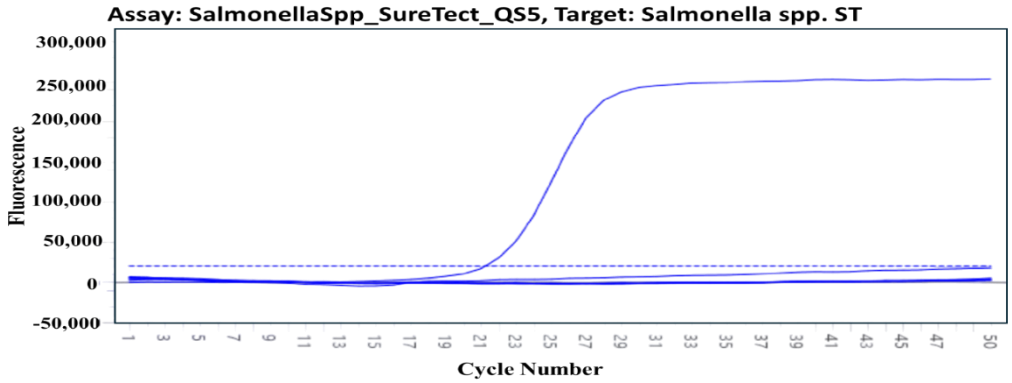

Figure (4): *Salmonella sp.* detection in cecal digesta by qPCR

Bacterial infections continue to be a primary worldwide health concern, contributing to morbidity and mortality in both humans and animals. The excessive use of conventional antibiotics in human, veterinary, and agricultural medicine has resulted in antimicrobial resistance. The escalating rates of resistance have spurred the exploration of alternative antimicrobial solutions, particularly in poultry production.

Our study revealed that adding different amounts of PHM to the diet notably increased the relative weight of the Bursa of Fabricius, especially at the 5% PHM level. However, it did not affect other organs, such as small intestine length, ceca, AWG, or AFI. Chickens treated with PHM exhibited slightly lower mortality under heat stress, though this difference was not statistically significant. Furthermore, supplementing broiler diets with PHM and trace elements at varying levels did not significantly change liver enzyme activity, cholesterol, HDL, LDL, glucose, or uric acid levels compared to the control. However, the 5% TE group significantly decreased triglyceride and VLDL levels.

Table (10): Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on serum biochemical parameters in broiler chickens exposed to chronic heat stress from 1 to 40 days

Dietary treatments		Cholesterol	HDL	Triglyceride	VLDL	LDL	AST	ALT	ALP	Uric acid	Glucose
PHM level	TE level										
0%	0 g/kg	125	52.6	87.6	17.5	54.5	294	4.46	3080	5.82	136
0%	0.5 g/kg	113	59.3	63.8	12.8	41.0	315	6.43	2986	5.40	193
2.5%	0 g/kg	104	56.3	66.0	13.2	34.5	279	5.07	2338	4.19	220
2.5%	0.5 g/kg	130	59.7	48.7	9.75	60.8	433	6.07	1915	5.24	236
5%	0 g/kg	106	52.8	76.2	15.2	37.7	346	8.32	2096	5.92	227
5%	0.5 g/kg	98	45.1	62.5	12.5	40.1	298	6.60	2093	4.00	215
Polyherbal Mixture level											
0% PHM		119	55.9	75.7	15.1	47.7	304	5.44	3033	5.61	214
2.5% PHM		117	58.0	57.4	11.5	47.6	356	5.57	2127	4.72	223
5% PHM		102	49.0	69.4	13.9	38.9	325	7.46	2095	4.96	221
Trace elements level											
0 g/kg		112	53.8	77.5 [a]	15.5 [a]	43.2	305	5.84	2549	5.35	228
0.5 g/kg		114	55.1	58.8 [b]	11.7 [b]	46.8	350	6.37	2382	4.92	211

Source of variation	Probability									
PHM	0.2175	0.0684	0.1433	0.1433	0.4902	0.7380	0.3674	0.0897	0.5514	0.7330
Trace elements	0.7939	0.7923	0.0228	0.0228	0.4587	0.4656	0.7447	0.6598	0.5466	0.3696
PHM X Trace Elements	0.1568	0.1578	0.8467	0.8467	0.0689	0.3788	0.4730	0.9024	0.2738	0.2588
Pooled SEM	4.36	1.66	4.10	0.82	3.52	27.0	0.61	195	0.34	7.00

Abbreviations: PHM; polyherbal mixture, TE; Trace elements; SEM; sum of standard errors mean, HDL; high density lipoprotein, VLDL; very low-density lipoprotein, LDL; low density lipoprotein, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase.

[a-c.] Means with different latter in the same column significantly differ ($P<0.05$).

Table (11): Effects of Polyherbal mixture (PHM) and trace elements (TE) supplementation on intestinal histomorphology in broiler chickens exposed to chronic heat stress from 1 to 40 days

Dietary treatments		Duodenum				Jejunum				Ileum			
PHM level	TE level	VL	CD	VL:CD	VSA	VL	CD	VL:CD	VSA	VL	CD	VL:CD	VSA
0%	0 g/kg	1568	222	7.19	0.194	968	145	6.71	0.110	705	171	4.20	0.081
0%	0.5 g/kg	1105	195	5.72	0.165	878	178	5.01	0.107	620	149	4.17	0.074
2.5%	0 g/kg	1422	192	8.00	0.186	875	151	5.92	0.078	638	140	4.50	0.067
2.5%	0.5 g/kg	1082	203	5.79	0.136	986	146	6.99	0.131	594	159	3.89	0.068
5%	0 g/kg	1297	173	7.69	0.130	922	152	6.02	0.108	617	142	4.45	0.073
5%	0.5 g/kg	933	182	5.03	0.119	704	176	4.06	0.087	516	163	3.26	0.070

Polyherbal Mixture level												
0% PHM	1362	210	6.54	0.181 [a]	928	160	5.95	0.108	667	161	4.19	0.077
2.5% PHM	1272	197	7.02	0.164 [ab]	924	149	6.39	0.104	616	150	4.19	0.067
5% PHM	1095	178	6.21	0.124 [b]	798	165	4.90	0.096	566	153	3.86	0.071
Trace elements level												
0 g/kg	1439 [a]	197	7.62 [a]	0.173 [a]	922	149	6.24	0.099	656	152	4.38	0.073
0.5 g/kg	1032 [b]	193	5.48 [b]	0.139 [b]	856	166	5.35	0.108	578	157	3.79	0.071
Source of variation	----------Probability----------											
PHM	0.2107	0.3400	0.8156	0.0087	0.6357	0.6168	0.2494	0.8199	0.4993	0.8078	0.7759	0.5457
Trace elements	0.0008	0.8965	0.0091	0.0342	0.5461	0.2385	0.2023	0.4908	0.2403	0.6650	0.1613	0.7298
PHM X Trace Elements	0.8682	0.5828	0.8016	0.4773	0.4665	0.4873	0.1294	0.1035	0.9300	0.3669	0.5635	0.9016
Pooled SEM	62.6	8.23	0.39	0.01	50.3	6.64	0.35	0.01	30.4	6.50	0.20	0.01

Abbreviations: PHM; polyherbal mixture, TE; Trace elements; SEM; sum of standard errors mean, VL; villus length, CD; crypt depth, VL:CD, villus length to crypt depth, VSA; villus surface area.

[a-c.] Means with different latter in the same column significantly differ ($P<0.05$).

Additionally, a PHM extract displayed potent antibacterial activity against *E. coli 0157:H7* and *Salmonella sp.* bacteria at a concentration of 20 mg. According to GC-MS analysis, the primary components identified in the PHM extract are linoleic acid, oleic acid, palmitic acid, and elaidic acid, listed in order of abundance. These compounds are classified as monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), which play a role in modulating immune system activity [37, 38]. Puthpongsiriporn and Scheideler [39] found that varying dietary levels of linoleic acid had no significant effect on poultry performance. In contrast, Al-Bandr and Qasim [40] studied the effects of conjugated linoleic acid on broiler growth and productivity. They found that linoleic acid at 1.5% and 2% levels improved performance, but its effect was not noticeable in other age periods. Consequently, in terms of performance, both studies are consistent with our findings.

The PHM improves metabolic efficiency, alleviates physical stress, inhibits cytokine release from macrophages, and enhances antimicrobial effects, leading to stronger immunity [41, 42]. According to Lelešius et al. [43], plant-based extracts provide antioxidant and antimicrobial benefits in poultry and reduce disease incidence in chickens. Additionally, Moraes et al. [44] demonstrated that supplementing linoleic acid strengthens the humoral immune response in broiler chickens. According to the results, adding PHM at varying levels reduced mortality compared to the control group, with the lowest mortality rates observed at 2.5% and 5% levels of supplementation. These levels decreased mortality from 18% (control) to 14%. This improvement may be attributed to PHM's antimicrobial and anti-inflammatory effects. Linoleic acid and oleic acid, both PUFAs with antimicrobial properties, may enhance immunity by increasing gene expression linked to interferon-mediated signaling pathways [45].

Garlic's characteristic smell is primarily due to organosulfur compounds, its main bioactive components. When garlic is crushed, chopped, or minced, alliin is rapidly converted into allicin (diallyl thiosulfate) by the enzyme alliinase [46]. Allicin exerts its effects by reacting with cellular thiol groups, such as l-cysteine and glutathione, producing S-allyl-mercapto-cysteine and S-allyl-mercapto-glutathione. These interactions may induce significant structural modifications in pathogen proteins, as Borlinghaus et al. [47] noted. Garlic contains various non-sulfur compounds such as lectins, polysaccharides, flavonoids, steroids, saponins, vitamins, allicin, fatty acids, minerals, and amino acids, which can act as synbiotic additives alongside organo-sulfur compounds [48]. Studies by Alagawany et al. (2021) and Melguizo-Rodríguez et al. [49] indicate that allicin and its derived organo-sulfur compounds exhibit antimicrobial, immunostimulatory, anti-inflammatory, and antioxidant properties, as well as other pharmacological benefits. Additionally, multiple studies have documented the beneficial impact of garlic and its extracts in combating microbial infections in poultry.

Peppermint, which has been used for medicinal purposes throughout history, is another important ingredient in PHM. It is rich in bioactive compounds, including menthol, menthone, carvacrol, limonene, 1,8-cineole, cineole, and α-pinene. These substances exhibit strong antimicrobial properties, effectively suppressing pathogens such

as *Escherichia coli, Staphylococcus aureus, Salmonella enteritidis, and Candida albicans. Moreover*, they function as coccidiostats, disrupting Eimeria species' different developmental stages, thereby enhancing poultry health and productivity [50-52]. The antimicrobial efficacy of peppermint essential oil has also been confirmed by.Thyme is another medicinal herb known to enhance appetite and feed consumption in poultry. It also boosts the production of natural digestive enzymes and supports immune function, thanks to its phenolic compounds [53]. The key bioactive components in thyme, thymol, and carvacrol, are responsible for its therapeutic effects, including antioxidant, antimicrobial, antiviral, and flavor-enhancing properties [54]. While some research indicates that thyme supplementation improves poultry performance [55, 56], other studies have found no significant impact [57]. Rosemary's active compounds provide beneficial properties such as antimicrobial, antioxidant, and anti-inflammatory effects. Additionally, its unique fragrance helps mask fishy odors while effectively slowing meat microbial growth and lipid oxidation [58-60].Pomegranate peel contains high levels of phenolic compounds, making it a more potent antioxidant than other parts of the fruit. Additionally, it possesses antifungal, antiparasitic, and anticancer properties [61]. Similarly, black cumin seeds are rich in bioactive compounds with antiviral and antimicrobial effects. Consistent with our findings, multiple studies have shown that incorporating black seed or its extracts into chicken diets boosts their immune response [62, 63]. The antimicrobial properties of these herbal medicines' components contribute to a powerful antimicrobial effect, enhancing chicken health and reducing mortality—all without negatively impacting growth performance or internal organ function.

Vitamins E and C and minerals like selenium, copper, and zinc are essential in boosting broiler chickens' performance under heat stress. They strengthen antioxidant defenses and immune responses, counteracting the negative impacts of high temperatures. These nutrients also support consistent feed consumption and growth while minimizing oxidative damage, leading to improved health and productivity. Moreover, they positively alter gut microbiota by fostering beneficial bacteria, which enhances nutrient uptake and gut health, increasing resistance to environmental stress [64].

In conclusion, several potent bioactive components in the PHM provide antibacterial properties against *E. coli* and *Salmonella sp. It reduces* both pathogens in the cecal content of chickens reared under chronic heat stress conditions. Also, incorporating varying levels of PHM notably enhanced the feed intake and was associated with slightly lower mortality rates in heat-stressed chickens. Similarly, adding PHM and trace elements at different concentrations to broiler diets did not significantly affect liver enzymes, cholesterol, HDL, LDL, glucose, or uric acid levels compared to the control. Therefore, PHM could be used as an antibacterial agent against both *Salmonella sp.* and *E. coli* species in chickens to reduce microbial resistance.

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