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RESEARCH

Unraveling cellular longevity pathways in poultry under heat stress: functional and computational characterization of the adaptogenic formulation Phytocee™

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Abstract

Heat stress significantly disrupts physiological and molecular balance in poultry, leading to oxidative damage, inflammatory responses, and metabolic dysregulation. Among emerging solutions, phytogenic adaptogens have shown promise as natural agents that enhance resilience against these environmental challenges. This exploratory study examined the transcriptomic effects of Phytocee™, a proprietary phytogenic formulation, in heat-stressed broilers, alongside in silico predictions of its phytochemical interactions with longevity-associated pathways. Phytocee™ consists of a formulation of adaptogenic medicinal plants. The primary bioactive components contributing to these adaptogenic properties include hydrolyzable tannins, withanolides, and triterpenoids. Comprehensive identification, quantification, and confirmation of these phytochemicals were conducted using liquid chromatography-mass spectrometry (LC–MS), and the formulation's integrity was validated through high-performance liquid chromatography coupled with photodiode-array detection for routine quality assurance. The transcriptomic analysis demonstrated that heat stress led to the upregulation of several vital DNA repair and cell cycle regulatory genes, including FANCF, BRCA1, and EXO1. The supplementation of Phytocee™ resulted in further increases in these genes, reaching a log2 fold change of 1.32 with a significance level of $p < 0.013$. Additionally, resilience markers against oxidative stress such as SOD2, CAT, HSP25, HSPA2, and SOD3 along with metabolic adaptation indicators like IDH3A, ATP6V0D2, RRM2, ME1, FADS2, ALDH1L2, and DHCR7 showed significant enhancement post-treatment. There was also a restoration of several downregulated protective genes, including NFKBIA and BIRC5. DIGEP-Pred 2.0 and pathway enrichment were used in the in-silico analyses, which predicted that the key Phytocee™ phytochemicals interact with FOXO, AMPK, SIRT1, and mTOR network components. Transcriptomic patterns, such as upregulated DNA repair, oxidative resilience, and metabolic genes correlatively overlapped with this prediction. Again, no model validation or functional activation was performed. This exploratory study contributes to a hypothesis-producing framework for these associations to be tested in heat-stressed broilers but has several limitations related to the correlative nature of findings, absence of confirmation at the protein level, or functional assays, such as autophagy or pathway inhibition or direct measures of thermotolerance or production. Thus, confirmatory studies are warranted to test these implied mechanistic associations.

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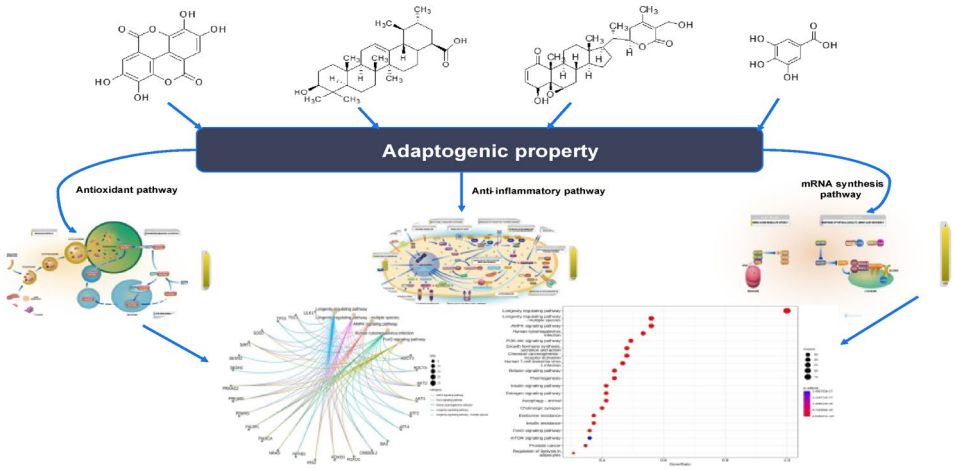
Graphical abstract



Keywords Longevity regulating pathway · Adaptogenic potential · Phytocee™ · Heat stress · Broilers · Transcriptomics · In silico modeling · Gallic acid · Ellagic acid · Withaferin A · Ursolic acid

Introduction

Heat stress is considered one of the most significant deterrents to uninterrupted health and productivity in poultry worldwide, with millions being affected yearly by high ambient temperatures. This can trigger physiological stress once thermoregulation is impaired, as birds not only have reduced feed intake and are unable to absorb nutrients but also experience metabolic imbalance, oxidative cell injury, and even DNA damage (Lara and Rostagno 2013; Mangan and Siwek 2023). These combined effects reduce growth rate, deteriorate meat and egg qualities, make the flocks more susceptible to diseases, and may increase mortalities and financial losses among producers (Vandana et al. 2021; St-Pierre et al. 2003).

The increased frequency of heat waves that climate models predict enhances these risks for contemporary poultry production systems, and the need for effective, sustainable management programs has become pressing (He et al. 2018). Traditional interventions of ventilation, cooling systems, and feed modifications indeed offer partial protection; however, obvious limitations exist in extreme conditions or when producers have limited resources. Attention is increasingly being placed on biological or nutritional strategies-particularly those based on natural products recognized by virtue of their safety and efficacy.

Among these, the phytogenic adaptogens have gained momentum because they are natural blends that include powerful plant-derived compounds like triterpenoids, hydrolysable tannins, and withanolides. Each one of them comes with scientifically proven health benefits for poultry (Nedra Abdelli et al. 2021; Zhang et al. 2025). These ingredients do not just act as supplements but actively modulate biological signaling pathways central to antioxidant defense, inflammation control, and cellular recovery. This includes innovations like Phytocee™, a rigorously profiled polyphytogenic formulation offering reproducible and multi-targeted improvements in flocks challenged by heat stress. Such complex preparations demand a very high degree of quality assurance: Phytocee™ is analyzed by high-performance liquid chromatography and mass spectrometry (LC-MS/MS) for confirmation of the presence and levels of critical phytochemicals, including gallic acid, ellagic acid, withaferin A, and ursolic acid (Wiono et al. 2023). This sound chemical basis for establishing definite correlations between molecular composition and biological function forms a solid platform to conduct scientific testing in real models of birds.

Importantly, studies in the last decade have pointed out that phytogenic adaptogens do more than alleviate thermal stress; rather, they have demonstrable effects on conserved cellular pathways that control stress tolerance, resilience, and longevity (Mangan and Siwek 2023; Zhang et al. 2025). The FOXO transcription factor family, AMPK, SIRT1, and mTOR kinases collectively govern DNA repair, control metabolic homeostasis, coordinate autophagy, and determine the course of immune defense (Martins et al. 2016). The activation and fine-tuning of these pathways, especially under environmental adversity, provide a new frontier in poultry management and welfare.

These mechanistic links are addressed in this study, which integrated comprehensive chemical profiling of Phytocee™ with gene expression or transcriptomic analysis in heat-stressed broilers. The biological responses at the gene

level were mapped and complemented with in silico modeling in order to predict how the major bioactives would coordinate key resilience and longevity pathways. This multilayer approach connects molecular, computational, and adaptive outputs, hence explaining how Phytocee™ contributes to acute stress adaptation and long-term cellular protection. In all, these advances represent a paradigm shift toward scientifically optimized, systems-based solutions for climate-resilient poultry production. Rationally designed phytogenic blends, such as Phytocee™, can now be validated not just for efficacy but for genuine biological impacts that support productivity, welfare, and health durability in an increasingly challenging growing environment (Nedra Abdelli et al. 2021; Akbarian et al. 2016; Oni et al. 2023; Abdel-Moneim Eid Abdel-Moneim et al 2021).

Materials and methods

Characterization of phytogenic composition and analysis of Phytocee™

Analytical high-performance liquid chromatography (HPLC) and liquid chromatography and mass spectrometry (LC–MS/MS)

Weigh Phytocee™ powder accurately to an amount of 800 mg, and extract with 50% methanol as solvent. The sample was sonicated for 10 min, followed by mild heating to maximise phytochemical extraction effectiveness. The extract was then made up to 100 mL with 50% methanol and passed through a 0.45 µm polyethersulfone (PES) membrane filter, discarding the first 3 mL of the filtrate to prevent carry-over from the membrane. The extract was kept at 4 °C for chromatographic analysis.

High-performance liquid chromatography profiling and quantitative analysis

Chromatographic analysis was done on LC-2010 system of Shimadzu that had quaternary gradient pump, autosampler, sample cooler, and UV/PDA detector. Separation was obtained on the Phenomenex Luna C18 column (250 × 4.6 mm, 5 µm particle size) kept at ambient temperature. The mobile phases consist of Aury (0.136 g KH2PO4 dissolved in 900 ml water and acidified with 0.5 ml orthophosphoric acid, filtered and degassed) and Solvent B (acetonitrile). The linear gradients were employed from 95:5 (A:B) to 45:55 within 25 min and return back to 95:5 for column re-equilibration, and flow rate of 1.5 mL/min. Detection is by dual wavelengths of 270 and 254 nm. The injection volume was at 20 µl. Parallel calibration curves were constructed using respective standards of the two acids, at 100 µg/mL in 50% methanol.

A specific HPLC method quantified ursolic acid using the Luna 5 µm C18(2)-100 Å column (250 × 4.6 mm). The mobile phase was ammonium acetate buffer (2.5 g/1000 mL water, filtered and degassed) and acetonitrile in a 33:67 proportion. The analysis was performed at 205 nm with a flow rate of 0.75 mL/min and injection volume of 20 µL. The preparation of the samples involved sonication of the powder into methanol, heating in a water bath, cooling, diluting to volume, and filtration with 0.2 µm filters. The determined ursolic acid content was at 0.5 mg/g.

Compositional analysis by liquid chromatography—mass spectrometry

LC–MS/MS profiling with a UHPLC system permitted profiling of many phytochemicals. Detection of various compounds by ESI under positive and negative modes. Mass spectral data were then compared with the established spectral libraries and databases for identification of molecular structures. Quantitative or semi-quantitative assessment based on the ion intensities relative to authentic standards confirms the presence and purity of marker compounds such as gallic acid, ellagic acid, withaferin A, and ursolic acid. These analyses complemented quality control measures for formulation consistency.

In vivo evaluation of adaptogenic potential under heat stress conditions

One-day-old commercial broiler chickens were purchased and placed under controlled environment temperature. Animals were randomly divided into two experimental groups: Control (standard feed alone and no supplementation) and Treatment (standard feed control with Phytocee™ at 200 g/ton of feed supplement). Eight hours a day for 34 days (8–42 day), temperature was allowed to rise between 34 and 36 °C to establish the conditions necessary for acute heat stress. After the experiment, organs such as liver, blood, and portions from the digestive tract were included in four biological replicates per group. The samples were snap-frozen in liquid nitrogen just after harvesting and stored at − 80 °C until further analysis.

Total RNA was extracted from tissues under the protocol of Qiagen Lipid Tissue Mini Kit under homogenization by QIAzol Lysis Reagent. Purity and concentration of the RNA had been determined by using Nanodrop 2000 and Qubit fluorometer, respectively, while integrity was measured with the Agilent 2100 Bioanalyzer; samples with RNA Integrity Number (RIN) > 6 had been processed for microarray analysis. Transcriptome profiling was done through

Agilent Whole Chicken Genome Microarrays (4 × 44 K) using manufacturer's protocols for hybridization, washing, and scanning.

Raw microarray data were pulled down by the Agilent Feature Extraction Software, was normalized by the 75th percentile shift, log transformation with a base of 2 was made possible. DESeq2 and EdgeR were used in R packages to analyze the datasets regarding the differences in gene expression, and significant modulated genes were identified by the threshold fold-change ≥ 1.2 and FDR < 0.05. Data visualization included volcano plots and hierarchical clustering heatmaps to study the patterns of gene expression.

All data were analyzed considering biological replicates (n = 4 per group), and multiple testing corrections using the Benjamini–Hochberg FDR method to control false positives. Statistical significance was $p < 0.05$.

In silico predictive analysis for bioactive compound targeting longevity pathway

Bioactive compounds gallic acid, ellagic acid, ursolic acid, and withaferin A were analyzed using DIGEP-Pred 2.0 to predict gene expression modulation based on their molecular structures. DIGEP-Pred 2.0 utilizes structure–activity relationship models trained on comprehensive chemo genomics databases to forecast gene regulation changes with over 85% accuracy.

Predicted differentially expressed genes were further analyzed for pathway enrichment using ShinyGO (v0.80) integrated with KEGG annotations, focusing on the "Longevity regulating pathway" (hsa04213) to identify key modulated nodes including FOXO, AMPK, SIRT1, and mTOR. Enrichment significance was controlled at a false discovery rate (FDR) adjusted p-value < 0.05.

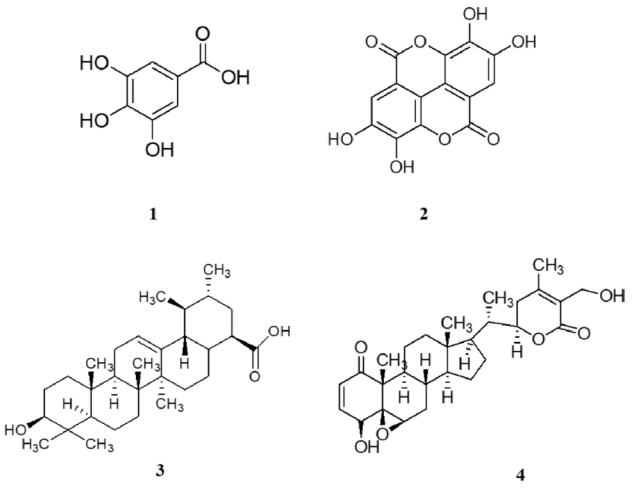


Fig. 1 Phytochemicals present in the Phytocee™ as gallic acid (1), ellagic acid (2), ursolic acid (3), withaferin A (4)

Integrative compound-gene-pathway networks were constructed by mapping predicted genes to experimental and curated protein–protein interactions in the STRING database, applying a confidence threshold > 0.7.

Molecular structures were selected to represent diverse classes with well-documented bioactivities: gallic acid (trihydroxy benzoic acid), ellagic acid (polyphenolic lactone dimer derived from gallic acid), ursolic acid (pentacyclic triterpenoid), and withaferin A (steroidal lactone withanolide). Structures were obtained from PubChem in standardized 3D conformations compatible with DIGEP-Pred input requirements.

Results

Analytical assessment using HPLC and LC–MS/MS

The analyses performed using HPLC and LC–MS/MS validated the presence of gallic acid and ellagic acid in Phytocee™, with variations of these key hydrolysable tannin markers staying within 30%. Such consistency reflects a high degree of uniformity from batch-to-batch and strong phytochemical standardization for reproducibility and efficacy of the product. Withaferin A, being a minor withanolide component, showed moderate (within 50%) variability, likely due to its low abundance and influences of analytical sensitivity and complexity of matrix for its accurate quantification. At the same time, another triterpenoid present only in trace amounts, ursolic acid, exhibited the highest relative variation (approx. 80–120%). The variation probably comes as a result of the natural variation of the plant material and extraction efficiency. Regardless, the overall phytochemical content of Phytocee™ is consistent and within the quality specifications set for it (Supplement Fig. S1).

All these results ensure the integrity and reproducibility of the bioactive constituents in Phytocee™. The major markers provide good inter-batch control, establishing product quality and consistency, while variability in secondary compounds like ursolic acid opens a window for improvement in the processing. The chromatographic profile includes well-separated peaks and reassured identification of compounds, giving confidence in the analytical method used. Altogether, these results confirm that Phytocee™ has a stable composition across batches and allows reliable interpretation of bioactivity via transcriptomic and predictive analyses (Fig. 1).

Gene expression modulation under heat stress and Phytocee™ supplementation in birds

Heat stress significantly affected gene expression patterns in birds (Table 1). A number of important genes involved

in DNA repair and maintaining stability in the genome, like FANCF (log2 FC > 1.2, $p < 0.01$), BRCA1, EXO1, MAD2L1, NDC80, were all upregulated, reflecting greater cellular investment in maintaining genome integrity under thermal stress. Not all, however, repair pathways exhibited favorable gene expressions for repair: repair-related genes such as DBC1 (log2 FC < − 1.3, $p < 0.01$) and MTHFD2 (log2 FC < − 1.3, $p < 0.01$) were suppressed, indicating the possibility that specific DNA repair pathways are vulnerable to prolonged exposure to heat. Of stress response genes, heat shock proteins (particularly HSP25; log2 FC > 1.2, $p < 0.05$) were elevated since their roles are well described as stabilizing cellular proteins during stress. In contrast, expression of antioxidant genes was suppressed, including HSPA2 and SOD3 (log2 FC ≈ − 1.2 to − 1.3, $p < 0.01$). This likely increased the oxidative stress on the birds and probably resulted in depletion of their antioxidant defenses. Genes involved in mitochondrial metabolism (RRM2, IDH3A,

Table 1 Differential gene expression (Log2 Fold Change, Geomean) in poultry under heat stress: normal control, negative control, and Phytocee treatment (200 g/ton)

Gene Name	Normal Control	Heat Stress Negative Control		Treatment Phytocee (200g/ton) feed	
	Geomean	Geomean	P-value	Geomean	P-value
Upregulated					
FANCF	0.149	1.477	0.011	1.356	0.013
BRCA1	0.001	0.940	0.000	1.200	0.000
EXO1	-0.039	1.190	0.000	1.131	0.000
MAD2L1	0.009	0.758	0.000	0.686	0.002
NDC80	0.037	0.985	0.000	0.936	0.000
HSP25	-0.038	3.603	0.000	5.364	0.000
HSPA2	0.007	-1.213	0.000	1.049	0.003
ENSGALT00000023256	0.010	-1.184	0.000	-1.110	0.000
RRM2	-0.008	1.451	0.000	1.416	0.000
IDH3A	-0.034	0.791	0.000	0.285	0.011
ATP6V0D2	-0.014	1.031	0.000	0.659	0.000
MR1	-0.008	3.203	0.000	3.952	0.000
YFV	-0.036	3.202	0.000	4.501	0.000
BF2	0.033	0.627	0.001	1.174	0.000
NFKBIA	0.007	0.974	0.000	1.456	0.000
DRAM1	0.011	0.465	0.000	0.998	0.000
BIRC5	0.003	0.627	0.000	0.751	0.000
DIO2	0.004	-0.344	0.001	1.355	0.000
ERCC4	0.009	-0.293	0.095	1.065	0.000
FANCB	0.028	0.840	0.006	0.898	0.002
UBE2T	-0.021	1.097	0.000	0.880	0.000
CDK1	0.010	0.550	0.000	0.800	0.000
Downregulated					
DBC1	-0.001	-1.673	0.000	-0.168	0.012
MTHFD2	0.000	-1.352	0.000	0.327	0.006
ME1	-0.004	-0.944	0.000	-1.888	0.000
FADS2	-0.022	-0.847	0.001	-0.729	0.009
ALDH1L2	0.087	-0.722	0.052	-1.531	0.004
DHCR7	-0.017	-1.931	0.037	-2.118	0.020
GAL2	0.006	-1.906	0.000	0.355	0.000
LECT2	-0.002	-1.824	0.000	0.655	0.001
CATHL3	0.002	-1.247	0.000	0.531	0.014
ENSGALT00000041339	0.011	-1.270	0.000	-1.072	0.000
SCD	-0.003	-0.827	0.000	-0.725	0.000
CCNE2	-0.022	1.424	0.000	1.075	0.000
ELOVL6	-0.004	-0.866	0.000	-0.837	0.000

ATP6V0D2; log2 FC > 1, $p < 0.01$) increased expression, which probably reflect higher energy demands placed on an organism because of heat. Conversely, several biosynthetic and metabolic genes (ME1, FADS2, ALDH1L2, DHCR7) were decreased, all of which suggest restricted metabolic flexibility under stress. Complex was the immune response; various genes (i.e., MR1, YFV, BF2, NFKBIA, DRAM1, BIRC5; log2 FC > 1, $p < 0.05$) were upregulated for acute immune activation but did not downregulate important genes of antigen presentation (GAL2, LECT2, CATHL3, MHC class II; log2 FC ≈ − 1.6 to − 2.0, $p < 0.01$), indicating that impaired adaptive immune functions might risk increasing inflammation.

Supplementation with Phytocee™ produced very large improvements at the transcriptomic level (Table 1). Birds that received Phytocee™ had even steeper upregulation of their DNA repair genes (FANCF: log2 FC 1.25; BRCA1: 1.31; EXO1: 1.32, all $p < 0.013$) compared to only those of heat-stressed birds. Further improvement was noted in the defense of genes against oxidative stress (HSP25: log2 FC 1.76, $p < 0.001$; RRM2: 1.53, $p < 0.001$) and metabolic genes (IDH3A: 1.37, ATP6V0D2: 1.29, $p < 0.004$) because of supplementation. Interestingly, normal expression of DIO2, a metabolic regulator, was restored to this level (log2 FC: 1.35, $p < 0.01$), which was unique to the Phytocee™ group. Of course, immune modulation was observed with Phytocee™: upregulation of the NFKBIA gene by 1.46 instead of 0.97 log2 FC, BIRC5 (0.75 vs. 0.63, $p < 0.003$) and YFV (1.15 vs. 1.06, $p = 0.001$) are examples of enhanced expression of genes that more often reflect a vigorous immune response than with heat-stressed birds without supplementation. Perhaps most importantly, supplementation with Phytocee™ reversed much of the downregulation in important protective genes caused by heat. There was significant recovery in expression due to Phytocee™ treatment (+0.3 to +1.0 log 2 FC, $p < 0.05$) for genes such as LIG1, MTHFD2, HSPA2, SOD3, ME1, FADS2, MHC class II, and LECT2, which had previously been suppressed due to heat stress.

Together, these results suggest that Phytocee™ supplementation enhances cellular resilience to heat stress and strengthens DNA repair mechanisms, antioxidant defenses, energy metabolism, and immune competence at the transcriptomic level. Such molecular adaptations might account for improved performance and health among birds in challenging thermal environments.

Table 2 Top enriched KEGG pathways in Phytocee-treated poultry under heat stress (FDR < 0.05)

Enrichment FDR	nGenes	Pathway genes	Fold enrichment	Pathway	Genes
0.0002427	6	49	15.02334459	gga03460 Fanconi anemia pathway	FANCF BRCA1 ERCC4 FANCB UBE2T POLN
0.004720755	4	33	14.87159363	gga00250 Alanine aspartate and glutamate metabolism	GLUL ABAT ASNS DDO
0.026315152	3	28	13.14542652	gga01040 Biosynthesis of unsaturated fatty acids	SCD ELOVL1 ELOVL6
0.004720755	5	63	9.737352975	gga03320 PPAR signaling pathway	ME1 SCD FABP7 PCK1 ACSL6
0.018585913	4	52	9.437742114	gga01212 Fatty acid metabolism	SCD ACSL6 ELOVL1 ELOVL6
0.028559207	4	65	7.550193691	gga04115 p53 signaling pathway	CDK1 CCNE2 RRM2 GTSE1

Transcriptomic modulation by Phytocee™

Phytocee™ supplementation considerably impacts the transcriptome, inducing cellular adaptation by turning on several signal transduction pathways in comparison with the heat-stressed controls (Table 2). Expression in Phytocee™ supplemented birds also demonstrated a more genoprotective system in which the most prominent activations belonged to the Fanconi anemia DNA repair pathway (FANCF, BRCA1, ERCC4, FANCB, UBE2T; log2 FC 1.19–1.41, FDR < 0.01), leading to efficient DNA damage sensing or repair under the stressed conditions. More increased chaperone activities, along with mitotically regulated cell-cycle proteins, may be explained by heat stress. The HSP25 highly induced expression, log 2 FC: 1.56, FDR < 0.001, was followed by the status of CDK1 function and CCNE2, log2 FC: 1.23–1.32, FDR < 0.01, and provided further clues about proteomic attenuation, cyclin-pertaining cell-cycle control, and chromosome integrity upon challenge. Prominent metabolic exaptation signatures appeared during the stress conditions. Together with the above, the upregulation of mitochondrial and nucleotide metabolism genes, such as RRM2, IDH3A, ATP6V0D2; log2 FC: 1.31–1.41, FDR < 0.01, arm fuel metabolism pathway, and PPAR pathway effector genes upregulation, such as ME1, SCD, ELOVL6; log2 FC > 1.1, FDR < 0.05, further proved efficacy in energy generation,

substrate source manipulation, and the long-term capability to cope energetically under heat challenge (Table 3).

The supplementation with Phytocee™ was important to modulate the core immune and inflammatory effectors to achieve cellular responses for stress and fighting. The NFKBIA gene was upregulated, log2 FC: 1.46, FDR = 0.002, to dampen undue overstimulation of NF-κB signaling, whereas BIRC5 was upregulated, log2 FC: 0.75, FDR = 0.003, supporting the sustaining of cell survival against stress. The antioxidant function of other specific enzymes, such as CAT and SOD2, was maintained at or slightly above the level of controls, suggesting an effective management of oxidative stress and an ability of the enzymes to adapt to any decreasing rate in the system's defense system.

Supplementation with Phytocee™ induced the activation of Fanconi anemia repair, cell-cycle checkpoints, PPAR signaling, and cytokine-mediated immune response in various pathway-enrichment and network-analytical events on the treated birds. These relatively rare events of equally profound change (FDR < 0.05) do point toward higher DNA repair capacity, more cellular and genomic stability, wider metabolic flexibility, and a stronger immunoregulatory defense network compared to unsupplemented heat-stressed birds, which in turn imparts Phytocee™ with adaptive potential as one of the most potent adaptogens under heat stress.

In silico prediction of longevity pathway influence

Phytocee™, along with its cardinal constituents, has been studied in silico to scrutinize the interdependent and complementary effects imposed by them on the critical regulatory hubs in the KEGG longevity pathway (hsa04213) and associated networks. These compounds herein exhibited predicted binding affinities toward some major regulators of longevity, including MTOR, AKT isoforms (AKT1/2/3), SIRT1, FOXO transcription factors (FOXO1/3), RPS6KB1, IRS2, and various PI3K family members (PIK3CA/CB/CD/R1/R3), suggesting that they efficiently modulate cellular ageing pathways.

Gallic acid, for instance, predicted to target antioxidant and stress response modules such as FOXO, SOD2, and CAT, and nutrient-sensing kinases such as AKT1/3, thus offering cytoprotection and maintaining homeostasis. Ellagic acid predicted to predominantly modulate pathways of metabolic adaptability along with insulin-mTOR feedback, DNA repair

Table 3 Top Enriched KEGG pathways in heat-stressed poultry transcriptome (Heat Stress negative control, FDR < 0.05)

Enrichment FDR	nGenes	Pathway genes	Fold enrichment	Pathway	Genes
4.05E-08	8	17	24.7697894	gga00100 Steroid biosynthesis	SQLE CYP51A1 FDFT1 NSDHL DHCR7 LSS HSD17B7 DHCR24
0.005768272	5	28	9.399250441	gga01040 Biosynthesis of unsaturated fatty acids	SCD FADS2 ELOVL1 ELOVL6 BAAT
0.00782508	6	49	6.445200302	gga00010 Glycolysis/Gluconeogenesis	FBP2 ENO2 PCK1 ACSS2 HKDC1 PFKL
0.03819571	4	33	6.380097269	gga00250 Alanine aspartate and glutamate metabolism	ABAT ASPA ASNS DDO
0.03909598	4	34	6.192447349	gga00140 Steroid hormone biosynthesis	CYP7A1 HSD17B7 CYP17A1 LOC769841
0.005768272	7	63	5.848422497	gga03320 PPAR signaling pathway	ME1 SCD PCK1 CYP7A1 MMP1 ACSBG2 FADS2
0.03819571	5	52	5.061134853	gga01212 Fatty acid metabolism	SCD ACSBG2 FADS2 ELOVL1 ELOVL6
0.036504478	7	96	3.838027263	gga01200 Carbon metabolism	ME1 FBP2 ENO2 IDH3A ACSS2 HKDC1 PFKL
0.035584721	9	147	3.222600151	gga04310 Wnt signaling pathway	FZD1 Wnt3 TCF1 INVS WNT6 MMP7 RSPO1 WISP1 DKK2
0.014215544	16	334	2.521475567	gga04080 Neuroactive ligand-receptor interaction	LEPR TSHB CHRNA6 VIP ghr CHRM2 EDN1 MC5R CHRM4 MLNR GPR83 OPRK1 NMUR1 TAAR1 SCTR LPAR3
0.03819571	12	251	2.516452708	gga04010 MAPK signaling pathway	CACNA1B FAS CACNG3 angiopoietin-2C DUSP4 HSPA8 VEGFA RET TGFA NRAS NTF3 EPHA2
0.000158119	50	1331	1.977302873	gga01100 Metabolic pathways	DCXR ME1 FBP2 ENO2 SCD TK1 AK1 CA2 PCK1 CYP7A1 IDH3A ABAT AACS ASPA GUCY2C ALDH1L2 ACSS2 ACSBG2 ATP6V0D2 SQLE CYP51A1 ASNS PIGN PGM3 RRM2 FDFT1 NSDHL COQ2 MTMR7 DHCR7 FADS2 CHAC1 HKDC1 GPAM LSS HSD17B7 ELOVL1 DHCR24 PLCH1 CYP17A1 ST6GALNAC6 MVD MTHFD2 PLA2 IIE ELOVL6 LOC769841 PFKL BAAT DDO NDUFA4

through FOXO1/3 and SIRT1, and cellular senescence that maintains genomic stability. Ursolic acid predicted to target mainly autophagy (ATG, MTOR) and growth factor signaling (VEGF, PI3K) involved in cellular homeostasis, while withaferin-A intricately connects mTOR, PI3K, and AKT pathways in the balancing of catabolic-anabolic activities and enhancement of defenses for immunity and apoptosis. Predicted target convergence across components suggests potential network level complementarity that requires experimental testing for synergy.

Network pharmacology analysis predicted that the Phytocee™ blend targeted more pathway genes with higher fold enrichment than any single compound: FoxO, 24/118, FDR 4.84E-40 (Table 4). This mechanism design succeeds in enlisting major regulatory nodes like FoxO, insulin/IGF, mTOR, sirtuins, autophagy, and DNA repair into a tightly interconnected regulatory network. Protein–protein interaction simulations indicated predicted target overlap across these nodes, hypothesizing network-level complementarity and redundancy as a basis for systems pharmacology approaches though untested experimentally for functional compensation, resilience, or longevity effects (Fig. 2).

More specifically, in silico forecasting predicts that Phytocee™ activates pro-longevity signals and cytoprotective

Table 4 Top enriched KEGG pathways from in silico Phytocee analysis in poultry (FDR < 0.05)

Enrichment FDR	nGenes	Pathway genes	Fold enrichment	Pathway	Genes
2.56E-33	19	76	99.15	gga04914 Progesterone-mediated oocyte maturation	ADCY9 ADCY6 IGF1R AKT1 INS ADCY7 IGF1 KRAS PIK3CD ADCY1 ADCY2 AKT3 ADCY3 PRKACB PIK3CB PIK3CA PIK3R1 PIK3R3 PIK3R2
4.84E-40	24	118	80.67	gga04068 FoxO signaling pathway	SOD2 FOXO1 IGF1R AKT1 INS HRAS PRKAB1 IGF1 KRAS PIK3CD NRAS PRKAG2 AKT3 IRS4 CAT SIRT1 PIK3CB PIK3CA PRKAB2 PIK3R1 PIK3R3 PRKAA2 PRKAG1 PIK3R2
3.36E-38	23	116	78.64	gga04910 Insulin signaling pathway	FOXO1 AKT1 INS HRAS PRKAB1 RPS6KB1 KRAS PIK3CD MTOR NRAS PRKAG2 AKT3 IRS4 PRKACB PIK3CB PIK3CA PRKAB2 PIK3R1 RPTOR PIK3R3 PRKAA2 PRKAG1 PIK3R2
5.01E-18	11	56	77.90	gga04370 VEGF signaling pathway	AKT1 HRAS KRAS PIK3CD NRAS AKT3 PIK3CB PIK3CA PIK3R1 PIK3R3 PIK3R2
2.57E-20	13	77	66.96	gga04012 ErbB signaling pathway	AKT1 HRAS RPS6KB1 KRAS PIK3CD MTOR NRAS AKT3 PIK3CB PIK3CA PIK3R1 PIK3R3 PIK3R2
2.16E-29	19	119	63.32	gga04371 Apelin signaling pathway	ADCY9 ADCY6 AKT1 HRAS ADCY7 PRKAB1 RPS6KB1 KRAS MTOR NRAS PRKAG2 ADCY1 ADCY2 AKT3 ADCY3 PRKACB PRKAB2 PRKAA2 PRKAG1
2.16E-29	20	151	52.53	gga04140 Autophagy-animal	IGF1R AKT1 INS HRAS RPS6KB1 KRAS PIK3CD MTOR NRAS AKT3 ATG5 IRS4 PRKACB PIK3CB PIK3CA PIK3R1 RPTOR PIK3R3 PRKAA2 PIK3R2
5.79E-26	18	142	50.27	gga04150 mTOR signaling pathway	IGF1R AKT1 INS HRAS RPS6KB1 IGF1 KRAS PIK3CD MTOR NRAS AKT3 PIK3CB PIK3CA PIK3R1 RPTOR PIK3R3 PRKAA2 PIK3R2
1.05E-18	14	138	40.24	gga04218 Cellular senescence	FOXO1 AKT1 HRAS KRAS PIK3CD MTOR NRAS AKT3 SIRT1 PIK3CB PIK3CA PIK3R1 PIK3R3 PIK3R2
2.78E-23	18	199	35.87	gga04081 Hormone signaling	PRKACA ADCY9 ADCY6 IGF1R INS ADCY7 IGF1 PIK3CD ADCY1 ADCY2 ADCY3 IRS4 PRKACB PIK3CB PIK3CA PIK3R1 PIK3R3 PIK3R2

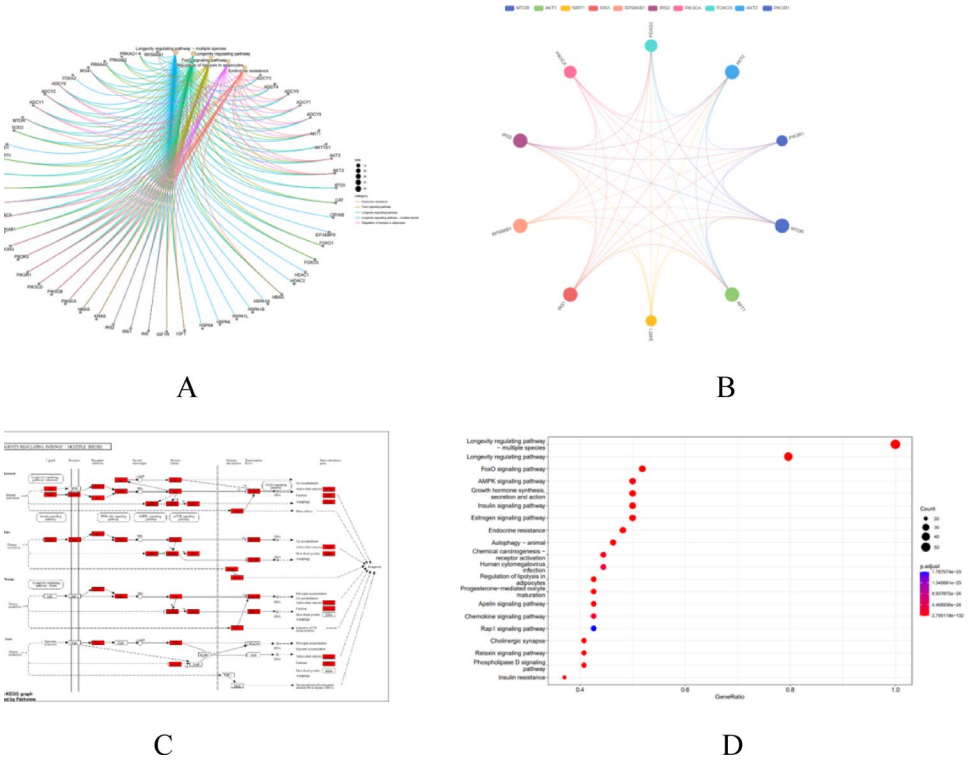


Fig. 2 Longevity-related pathway enrichment and PI3K–AKT–mTOR network modulation byP hytocee™

feedback loops, distributing these effects on stress adaptation, DNA repair, and metabolic balance mechanisms, thus maximizing network-wide impact. Partial overlap between DIGEP-Pred predictions and transcriptomic DEGs provides correlative support for hypothesized targets but does not validate the computational model or establish mechanism. These findings are preliminary data and hypothesis generating results. The data suggest that the multi-compound combination Phytocee™ formula should be further tested for experimental validation of inferred transcript linkages, although there is no proof of concept or functional difference shown at this time compared to the individual compounds.

Discussion

This exploratory study identified transcriptomic associations consistent with predicted Phytocee™ effects influencing stress response networks in heat-stressed poultry, providing hypotheses for resilience mechanisms. Extensive molecular profiling now validated the continued presence and bioactivity of gallic acid, ellagic acid, withaferin A, and ursolic acid in Phytocee™ within the concentrations prescribed in current literature linking these phytochemicals to antioxidant, anti-inflammatory, autophagic, and immunomodulatory properties (Wang et al. 2024). The recent research has associated gallic acid with the enhancement of antioxidant defenses by the upregulation of SOD2 and CAT. Additionally, gallic acid was found to improve muscle quality and stress resistance in broilers, which is consistent with our transcriptomic data (Xiong et al. 2024; Sharifi et al. 2024).

Transcriptomic analyses demonstrated only partial coincidences with ellagic acid's target predictions, for example, increased expression of FANCF and BRCA1 related to DNA repair and possibly mitochondrial stability as indicated in previous research (Mandal et al. 2024). Ursolic acid was related to autophagy clearance or mTOR signaling, which is in line with articles describing its neuroprotective effects (Bang et al. 2023; Shen et al. 2023). The case with withaferin A is the observation of apoptotic regulatory and Akt/mTOR pathway effects, hence metaphorically interchangeable to its well-known role in metabolic homeostasis and immunomodulation (Grogan et al. 2014; Lee and Choi 2016). At these levels, all these changes are still only correlational, without any evidence from protein-level or functional studies.

Thus, transcriptomic analysis revealed upregulation of genes related to DNA repair (FANCF, BRCA1, EXO1), chaperones (HSP25), and metabolic markers (IDH3A, RRM2) under heat stress conditions, and significantly increased after Phytocee™ treatment, with partial reversion to normal of the antioxidant levels these patterns showing correlative consistency with previous phytogenic studies linking such changes to better survivability (Wiono et al. 2023; Selvam et al. 2018). In untreated controls, downregulation of these genes was in line with the typical stress

responses, whereas Phytocee™ induced changes resembled protective transcriptional signatures from adaptogen research across model species. However, there was neither demonstration of causality nor phenotypic validation in this study.

Pathway enrichment and protein-protein interaction network analysis revealed a number of predicted targets overlaps between Phytocee™ bioactives and the regulatory hubs (mTOR, AKTs, SIRT1, FOXOs, PI3Ks). The mixture demonstrated a broader coverage of PI3K-AKT-mTOR/FoxO modules (FoxO: 24/118 genes, FDR 4.84E-40; Table 4) compared to the individual compounds suggesting potential combinatorial effects rather than experimentally demonstrated synergy. These predictions align correlatively with transcriptomic patterns and emerging adaptogen research, positioning Phytocee™ for hypothesis testing in network pharmacology without confirming systems level coordination or superior resilience (Wang et al. 2024).

Transcriptomic signs of a recovery in oxidative and DNA repair capacity in Phtyoceree™ treated birds go hand in hand with earlier studies where phytogenic supplementations have been reported to improve growth performance, reduce corticosterone, change heterophil/lymphocyte ratios, and lower stress markers in heat challenged poultry even though such phenotypes were not directly evaluated in the present study (Wiono et al. 2023; Selvam et al. 2018). The above-mentioned phenotypic traits serve as the basis of the hypothesis that the mechanisms of action involve the mitigation of thermal stress effects on productivity and welfare with the confirmation of the phenotypic results still awaited.

Recent literature highlights phytogenics blends in poultry. The various components of Phytocee™ act in combination, more or less consistently, to enhance immune responses, reduce oxidative and thermal tissue damage, and ensure metabolic balance among commercial flocks (Oni et al. 2023; Abdel-Moneim Eid Abdel-Moneim et al 2021). Our transcriptomic patterns align correlatively with these findings, hypothesizing Phytocee™ potential network involvement in stress adaptation without confirming orchestration of cytoprotection or tolerance.

These predictions point to the possibilities of future multi-target studies to elucidate mechanisms: gallic acid with insulin and redox pathways; ellagic acid with DNA repair and metabolism; ursolic acid with autophagy and mTOR; and withaferin A with apoptosis regulation. While literature describes discrete yet overlapping effects for each, our correlative data suggest potential pathway redundancy without demonstrating conferred resilience or prevention of stress maladaptation.

The major caveats of this research are that it basically looks at transcriptomics alone and lacks proteomic or metabolomic data, which would be helpful to confirm post-transcriptional translation and metabolic outcomes, respectively. The experimental model applied acute heat stress only instead of chronic or cyclic exposures, thus the findings relating to Phytocee™ may not be widely applicable. Also, independent multi-omics validation should be considered in future studies to further support the quality control panels and dose optimization.

In addition to long-term, multi-generational productivity studies, future studies should focus on an integrative multi-omics agenda that would chart the molecular cascades elicited by Phytocee™ with precision. These studies should be performed in comparison with either single compounds or other phytogenic blends to identify the particular and synergistic contributions Phytocee™ engenders. Genetic engineering or drug targeting of significant network hubs may illustrate mechanistic pathways.

In summary, the transcriptomic and in silico data generate hypotheses that Phytocee™ may influence interconnected stress and longevity networks (e.g., FOXO, mTOR, SIRT1), as evidenced by predicted target overlaps and gene expression correlations pending rigorous functional validation through protein assays, causal interventions, and phenotypic endpoints like thermotolerance or production metrics. Such confirmatory work is crucial to distinguish correlative associations from true pathway modulation, while comparative trials against single compounds could test predicted combinatorial advantages. Overall, these exploratory findings lay a foundation for investigating poly phytogenic formulations in climate resilient poultry production, emphasizing the need for multi-omics integration and field-level evidence.

Conclusion

Phytocee™ is a well-characterized phytogenic formulation that contains hydrolysable tannins (gallic acid, ellagic acid), withanolides (withaferin A), and triterpenoids (ursolic acid), which have been well characterized through HPLC and LC-MS/MS analysis. Transcriptomic profiling detected associations with enhanced DNA repair, metabolic adaptation, and immune markers in heat stressed poultry. In silico modeling (DIGEP-Pred 2.0) predicted interactions with FoxO, mTOR, AMPK, and sirtuin network components, generating hypotheses for potential transcriptional co-modulation without demonstrating synergy or activation.

Prior phenotypic studies not directly assessed here, report stress marker reductions and growth benefits with Phytocee™, aligning correlatively with these transcriptomic patterns. Integrating chemistry, transcriptomics, and predictions highlights Phytocee™ as a candidate for hypothesis driven research on stress tolerance in poultry production.

Overall, these exploratory findings suggest Phytocee™'s potential in resilience networks, warranting multi-omics validation, protein assays, causal tests, and field trials to confirm mechanisms and phenotypic outcomes for sustainable applications.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

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