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Nutritional and antibacterial properties of yeast-fermented herbs for functional poultry feeds

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ABSTRACT

This study assesses the nutritional and bactericidal characteristics of yeast-fermented herbs as prospective substitutes for antibiotics in animal feed. A fermentation process was conducted on five plants, namely *Syzygium polyanthum*, *Curcuma longa*, *Andrographis paniculata*, *Orthosiphon stamineus*, and *Euphorbia hirta*, using *Saccharomyces cerevisiae*. Analysis conducted after fermentation showed notable alterations in the nutritional composition, with a rise in protein content and a decline in carbohydrate levels. The mineral profiling revealed fluctuations in nutrient contents, particularly significant increases in nitrogen and phosphorus. The antibacterial assays showed that fermented herbs were more effective against bacteria isolates, *Escherichia coli*, *Streptococcus gallolyticus*, *Staphylococcus aureus*, and *Aeromonas hydrophila*, than non-fermented herbs. The study found that fermented *Curcuma longa* has a similar antibacterial effectiveness as tetracycline, making it a potential alternative antimicrobial agent in animal feed. The study reveals that yeast-fermented herbs might be a viable and efficient substitute for antibiotics in poultry production, while also being environmentally friendly.

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INTRODUCTION

There is a growing interest in finding alternatives to conventional antibiotics for sustainable and health-conscious livestock management techniques, especially in the poultry industry. In the last twenty years, there has been an increasing focus on utilising the capabilities of medicinal herbs in the practice of broiler chicken farming. The adoption of sustainable alternatives to antibiotics and other synthetic therapies is motivated by concerns about antibiotic resistance, environmental sustainability, and consumer desire for ethically produced poultry products (Pliego *et al.*, 2022).

Scientists are progressively acknowledging the wide range of bioactive substances present in medicinal herbs, including antibacterial, immunomodulatory, and anti-inflammatory properties (Gurjar & Pal, 2021). Farmers aim to reduce their reliance on synthetic drugs and improve the well-being of hens by including medicinal herbs into poultry meals or management practices. This aligns with the evolving customer preferences for poultry products that are produced in a responsible manner and do not contain artificial substances. Several pressing concerns, foremost among them the escalating threat of antibiotic resistance, drive the adoption of medicinal herbs (Saha & Sarkar, 2021; Singh *et al.*, 2021). The widespread use of antibiotics in poultry production contributes to the emergence of resistant bacteria and raises environmental and food safety concerns about antibiotic residues in poultry products (Selaledi et al., 2020).

In order to tackle these issues and maximise the utilisation of therapeutic herbs, scientists are investigating fermentation as a possible method. Fermentation techniques can improve the stability, bioavailability, and effectiveness of bioactive chemicals found in plants (Mapelli-Brahm *et al.*, 2020). Additionally, fermentation promotes intestinal health in poultry by modulating gut microbiota composition and function (Liu *et al.*, 2021). Fermented herbs can promote the development of a diverse and harmonious microbial community in the chicken gut. This, in turn, can enhance the absorption and digestion of nutrients, strengthen the intestinal barrier, and reduce the

proliferation of detrimental bacteria (Yaqoob *et al.*, 2021; Zhou *et al.*, 2022).

Nevertheless, there are still hurdles that persist, such as the diversity in the composition and efficacy of bioactive compounds present in various herbs. Further research is necessary to fully understand the impact of fermentation on medicinal herbs and its effects on the health and performance of chickens. Understanding the mechanisms of fermentation and its impact on chicken physiology would enable scientists to develop tailored fermentation techniques that optimise the utilisation of medicinal herbs in poultry farming.

The objective of this study is to assess the nutritional and antibacterial properties of herbs that have been fermented with yeast, with the intention of using them as a substitute for antibiotics in the poultry feeds.

MATERIALS AND METHODS

Collection of Herbs and Sample Preparation

The study utilised five herbs: *Syzygium polyanthum*, *Curcuma longa*, *Andrographis paniculata*, *Orthosiphon stamineus*, and *Euphorbia hirta*. With the exception of *E. hirta*, which was obtained in March 2023 from Share Farm 2 of Universiti Putra Malaysia Bintulu Sarawak Campus, Sarawak, Malaysia (3°12'30" N, 113°04'53" E), the remaining plant samples were bought at the nearby fresh market. The samples underwent a washing process using distilled water and were subsequently subjected to drying in an oven at a temperature of 60 °C for a duration of 72 hours. Once the drying process was complete, the substance was pulverised into a fine powder using an electric grinder. The resulting powder was then stored in containers that were sealed tightly to prevent air from entering, and kept at the temperature of the surrounding environment.

Fermentation Process

The herbal extracts of *S. polyanthum*, *C. longa*, *A. paniculata*, *O. stamineus*, and *E. hirta* underwent fermentation using the yeast, *Saccharomyces cerevisiae*. Each herb was infected using 50 g of powdered plant material and 25 mL of *S. cerevisiae* broth, with an initial count of 8.23 log CFU/mL. Additionally, 10 mL of molasses and distilled water were added to the inoculum. The mixture was then incubated in polyethylene bags at 28 °C for 14 days.

Determination of pH

The pH of the fermented plant extracts was measured three times at the beginning and end of the process. The samples were diluted with sterile distilled water and the pH was measured using a handheld pH metre (HM Digital). The mean of the initial and final pH measurements of the five fermented herbs are presented in Table 1. The pH of fermented herbs was recorded in the range of 4.07 to 5.03 for the initial pH and 4.27 to 4.50 for the final pH in the present study.

Nutritional Analysis

Analysing the nutritional composition of both fermented and non-fermented plant samples was done using the standard methods specified by the Association of Official Analytical Chemists. This study entailed determining the amount of ash present by the use of a muffle furnace for heating, measuring the quantity of crude fat using a Soxhlet device, and evaluating the level of crude protein using the micro-Kjeldahl technique. In addition, conventional biochemical techniques were used to measure the amounts of crude fibre, total soluble sugars, reducing sugars, non-reducing sugars, and total carbohydrates.

Mineral Profiling

The mineral content (N, P, Ca, K, Mg, Cu, Zn, Na) in each sample was analysed by Atomic Absorption Spectroscopy (AA 800, Perkin-Elmer, Rodgau, Germany) following digestion with a mixture of nitric acid and perchloric acid.

Herb Extraction

By employing 96% ethanol as an extraction solvent, we obtained plant samples that were both fermented and non-

Table 1: Nutrient composition of non-fermented and yeast-fermented herbs (g/100 g)

Herbs	Parameters					
	Carbohydrate (g)	Protein (g)	Fat (g)	Crude Fiber (g)	Ash (%)	Energy (kcal)
n-fermented						
C. longa	43.94±0.70^{b}	6.36±0.21^{cd}	2.51±0.17^{a}	15.10±0.39^{d}	12.78±0.13^{a}	253.99±11.62^{b}
S. polyanthum	53.21±0.36^{a}	3.44±0.16^{f}	2.37±0.12^{ab}	22.84±0.18^{c}	7.17±0.27^{c}	308.75±3.82^{a}
A. paniculata	43.96±0.40^{b}	5.69±0.08^{e}	1.38±0.20^{cd}	32.60±0.74^{a}	6.15±0.24^{d}	303.77±9.11^{a}
E. hirta	53.28±0.25^{a}	5.79±0.11^{de}	2.15±0.12^{b}	14.38±0.42^{d}	9.05±0.53^{b}	291.16±12.29^{a}
O. stamineus	42.99±0.43^{b}	6.42±0.20^{cd}	1.72±0.13^{c}	28.89±0.32^{b}	5.44±0.18^{e}	290.45±2.32^{a}
Yeast-fermented						
C. longa	26.54±0.38^{f}	10.14±0.03^{a}	1.57±0.03^{c}	8.70±0.44^{g}	7.62±0.00^{c}	147.15±0.31^{f}
S. polyanthum	36.66±0.47^{c}	6.59±0.02^{c}	2.27±0.11^{ab}	9.96±0.29^{f}	2.09±0.02^{g}	195.55±3.47^{c}
A. paniculata	24.77±0.03^{g}	6.61±0.13^{c}	1.12±0.02^{d}	13.19±0.19^{e}	12.72±0.07^{a}	156.33±0.73^{ef}
E. hirta	32.18±0.48^{d}	6.32±0.50^{cd}	1.20±0.02^{d}	10.21±0.14^{f}	3.47±0.02^{f}	174.35±2.36^{de}
O. stamineus	30.59±0.44^{f}	7.57±0.10^{b}	1.07±0.06^{d}	12.33±0.12^{e}	3.57±0.03^{f}	175.93±1.44^{d}

Data are expressed as Mean±SE and are the average of four replicates. Different letters within the same column are different at $p<0.05$

fermented. The samples were immersed, separated, and condensed using a rotating vacuum evaporator before being stored at -20 °C.

Bacterial Strains Used

This study utilized representative strains of both Gram-positive and Gram-negative bacteria. The selected reference strains *Escherichia coli*, *Streptococcus gallolyticus*, *Staphylococcus aureus*, and *Aeromonas hydrophila* were obtained from the Microbiology Laboratory at UPMKB, Sarawak, Malaysia. Each strain was subcultured to obtain pure isolates, which were then prepared for in vitro antimicrobial activity testing using Mueller-Hinton agar.

Inoculum Preparation

Inoculum preparation was performed using a modified Kirby-Bauer disk diffusion method. (Hudzicki, 2009) Active bacterial cultures were obtained by transferring a loopful of stock cultures onto Mueller-Hinton agar plates, followed by overnight incubation at 37 °C. A sterile cotton swab was used to evenly spread 0.5 mL of the bacterial suspension onto the surface of the agar plates. To standardize the turbidity of the bacterial suspension, colonies were selected and diluted in distilled water. The suspension was vortexed thoroughly to ensure a uniform mixture. The optical density of the suspension was then measured using a spectrophotometer (Varian Cary 50 UV-VIS, Australia) at 600 nm and adjusted to an absorbance of 0.132, which corresponds to the turbidity of a 0.5 McFarland standard. This standard represents an approximate bacterial concentration of 1.5×10^8 CFU/mL.

Antibacterial Activity Assay

The antibacterial activity of selected traditional medicinal plant extracts was evaluated using the agar disc diffusion method (Hudzicki, 2009). Extracts were prepared at concentrations of 50 mg/mL from a stock solution of 100 mg/mL. Sterile 5 mm discs were cut from Whatman No. 1 filter paper and soaked in each concentration of plant extract for 24 hours. After soaking, the discs were placed onto nutrient agar plates previously inoculated with selected bacterial strains. The negative control was a 10% herb extract that was prepared using 96% ethanol. The positive control was a Tetracycline disc (30 mg). The plates were incubated at 37 °C, and each concentration was tested in triplicate. Antibacterial activity was assessed by measuring the diameter of the inhibition zones around each disc. The effectiveness of each plant extract concentration was compared to both the positive and negative controls.

Statistical Analysis

Statistical significance at the 95% confidence level was evaluated using Duncan's multiple-range tests, and data analysis was conducted using SAS statistical software (version 9.4, SAS Institute Inc., NC, USA).

RESULTS AND DISCUSSION

Nutrient Composition

The current study conducted a comparative nutritional analysis of five distinct varieties of herbs, both non-fermented and fermented. The results of this analysis were found to be variable and are presented in Table 1.

The process of yeast fermentation led to a substantial decrease in the amount of carbohydrates present in all herbs. This occurs because the yeast metabolises carbohydrates to provide energy and facilitate development. As an example, the carbohydrate content of *Curcuma longa* reduced from 43.94 g/100 g to 26.54 g/100 g, whereas *Andrographis paniculata* decreased from 43.96 g/100 g to 24.77 g/100 g. Yeast cells utilize carbohydrates as their primary energy source. During fermentation, yeast metabolizes these carbohydrates through glycolysis, converting them into simpler compounds like alcohol and carbon dioxide (Bertels *et al.*, 2021; Carsanba *et al.*, 2021).

The protein content typically experienced an increase following fermentation, most likely as a result of the introduction of yeast biomass. After fermentation, *C. longa* had a significant rise in protein content, increasing from 6.36 g/100 g to 10.14 g/100 g. This makes it one of the herbs with the highest protein enrichment. *O. stamineus* had a significant rise from 6.42 g/100 g to 7.57 g/100 g. The increase in protein content in herbs after fermentation is primarily due to the introduction and proliferation of yeast biomass, which is protein-rich and contributes to the overall protein levels in the fermented herbs (Santos *et al.*, 2022).

The fat content exhibited minor fluctuations after the fermentation process. The fat content of Curcuma longa fell from 2.51 g/100 g to 1.57 g/100 g. The fat content of *S. polyanthum* remained largely constant, with a slight drop from 2.37 g/100 g to 2.27 g/100 g. The reduction in fat content in *C. longa* is likely due to the active metabolism of fats by fermenting microorganisms, while the relatively stable fat content in *S. polyanthum* indicates a lesser impact from fermentation. The specific microbial activity and the inherent properties of the plant materials play crucial roles in determining the outcomes of the fermentation process (Martina *et al.*, 2020; Buniowska-Olejnik *et al.*, 2023).

The fibre content of *Euphorbia hirta* decreased from 14.38 g/100 g to 10.21 g/100 g, whereas *O. stamineus* exhibited a decline from 28.89 g/100 g to 12.33 g/100 g. The observed decrease in crude fibre content in *E. hirta* and *O. stamineus* during fermentation is a result of the effective breakdown of fibre components by microbial enzymes, facilitated by the fermentation process. This transformation not only reduces fibre content but can also enhance the nutritional profile of the fermented products, making them more digestible and beneficial for consumption (Debi *et al.*, 2019). The ash content, which is an indicator of the total mineral composition, exhibited variation. The ash content of *A. paniculata* increased significantly from 6.15% to 12.72%,

indicating a higher concentration of minerals resulting from the decomposition of organic matter during fermentation (Olagunju *et al*., 2023). The energy content of all herbs was reduced after fermentation due to a drop in carbohydrate content. As an illustration, the energy content of *C. longa* decreased from 253.99 kcal/100 g to 147.15 kcal/100 g, indicating a substantial decrease in carbohydrates (Hor *et al*., 2022).

The process of yeast fermentation modifies the nutritional makeup of herbs, increasing their protein content while decreasing carbs and overall energy content. *C. longa* and *O. stamineus* were identified as the most effective herbs for fermentation based on their significant increase in protein content and modest loss in carbohydrates. This resulted in a more balanced nutritional composition. *C. longa* is notable for its elevated protein content after fermentation, which makes it a very suitable choice for functional food applications.

Mineral Composition

Table 2 displays the mineral makeup of five plants in both their non-fermented and yeast-fermented states. The minerals analysed comprise nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), copper (Cu), zinc (Zn), and sodium (Na), with nutritional levels represented as percentages (%) or parts per million (ppm).

The current investigation discovered that fermentation led to an augmentation in the nitrogen content of all herbs. The greatest concentration of *Curcuma longa* (3.26%) suggests an increased protein content resulting from fermentation. These findings indicate that yeast fermentation boosts the nitrogen levels, which could potentially improve the nutritional quality of the herbs. After fermentation, the phosphorus content of all herbs increased, with *C. longa* (0.49%) and *O. stamineus* (0.36%) having the highest values. The herbs' energy metabolism and bone health advantages can be enhanced by an increase in phosphorus content. The potassium level of *C. longa* was reduced to 5.14%, yet it remains the highest among the plants.

Several other herbs exhibited minor deviations, generally indicating a decrease in potassium levels after the fermenting process. These findings indicate that fermentation could result in a little release of potassium. The magnesium concentration of most herbs reduced significantly during fermentation, with *A. paniculata* and *C. longa* both measuring 0.41%. The decrease implies that a certain amount of magnesium may be lost during the fermentation process. The calcium concentration of certain herbs, such as *C. longa*, was reduced to 0.76%, whereas in others like *A. paniculata*, it grew to 2.40%. These findings suggest that the calcium concentration of herbs can be affected differently by fermentation, which may be impacted by the herb's original composition and the conditions of fermentation. The copper level often decreased during fermentation, with *A. paniculata* exhibiting a modest drop (8.54 ppm). The decrease in copper content could be attributed to its consumption by yeast during the process of fermentation. The zinc concentration in *A. paniculata* remained elevated at 128.50 ppm, suggesting a modest rise after fermentation. These findings indicate that zinc remains essentially unchanged during the fermentation process and may possibly become more concentrated. The sodium concentration of most herbs declined dramatically after fermentation, however, *E. hirta* still exhibited high levels, albeit lowered to 2082.25 ppm. The substantial reduction implies that sodium is probably extracted during the fermentation phase.

Antibacterial Activity

The antimicrobial activity of both fermented (F) and non-fermented (NF) herb extracts was assessed against various bacteria, including *E. coli*, *A. hydrophila*, *S. aureus*, and *S. gallolyticus* (Figure 1). The results show that fermented herb extracts generally exhibit higher antimicrobial activity compared to their non-fermented counterparts, as indicated by taller bars representing fermented extracts across all tested herbs.

Upon examining each herb, it was shown that the fermented extract of *A. paniculata* exhibited much more efficacy in eradicating all tested bacteria compared to the non-fermented extract, except *E. coli*. *C. longa* likewise showed an increase in the areas of inhibition for the fermented extract compared to the non-fermented herbs, except for *S. aureus*. The antibacterial activity of the fermented extract of *S. polyanthum* was substantially greater than that of the non-fermented extract.

Table 2: Mineral composition of non-fermented and yeast-fermented herbs (g/100 g)

Herbs	Nutrient elements							
	N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Cu (ppm)	Zn (ppm)	Na (ppm)
Non-fermented								
C. longa	2.31 ± 0.06^{cd}	0.33 ± 0.02^{c}	5.89 ± 0.25^{a}	0.44 ± 0.01^{b}	0.92 ± 0.11^{e}	6.74 ± 0.52^{e}	77.88 ± 0.56^{b}	308.00 ± 2.48^{def}
S. polyanthum	1.26 ± 0.09^{h}	0.23 ± 0.02^{fg}	1.78 ± 0.00^{e}	0.23 ± 0.01^{c}	1.54 ± 0.02^{d}	3.94 ± 0.13^{g}	55.26 ± 0.11^{d}	352.50 ± 2.10^{cd}
A. paniculata	1.14 ± 0.02^{h}	$0.21\pm^{gh}$	2.52 ± 0.03^{c}	0.52 ± 0.01^{a}	1.42 ± 0.07^{d}	9.07 ± 0.12^{a}	125.98 ± 2.10^{a}	373.00 ± 5.48^{c}
E. hirta	1.46 ± 0.05^{g}	0.15 ± 0.01^{i}	$2.17\pm^{d}$	0.24 ± 0.22^{c}	2.86 ± 0.99^{a}	8.17 ± 0.10^{bc}	72.05 ± 2.89^{c}	2365.75 ± 54.05^{a}
O. stamineus	1.62 ± 0.10^{f}	0.18 ± 0.01^{h}	2.19 ± 0.02^{d}	0.26 ± 0.02^{c}	0.97 ± 0.01^{e}	8.33 ± 0.10^{bc}	56.38 ± 0.52^{d}	324.50 ± 1.94^{cde}
Yeast-fermented								
C. longa	3.26 ± 0.01^{a}	0.49 ± 0.00^{a}	5.14 ± 0.01^{b}	0.41 ± 0.00^{b}	0.76 ± 0.01^{f}	6.04 ± 0.35^{f}	68.73 ± 0.37^{c}	255.00 ± 5.77^{f}
S. polyanthum	2.18 ± 0.01^{de}	0.25 ± 0.00^{ef}	0.78 ± 0.00^{f}	0.11 ± 0.00^{e}	0.97 ± 0.01^{e}	3.31 ± 0.03^{g}	45.43 ± 0.01^{e}	312.50 ± 4.33^{cdef}
A. paniculata	2.14 ± 0.01^{e}	0.28 ± 0.00^{d}	2.48 ± 0.00^{cd}	0.41 ± 0.00^{b}	2.40 ± 0.01^{b}	8.54 ± 0.23^{ab}	128.50 ± 5.21^{a}	330.00 ± 1.73^{cde}
E. hirta	2.43 ± 0.03^{bc}	0.27 ± 0.00^{de}	1.62 ± 0.01^{e}	0.19 ± 0.00^{c}	1.82 ± 0.00^{c}	7.22 ± 0.05^{de}	57.05 ± 0.07^{d}	2082.25 ± 28.72^{b}
O. stamineus	2.46 ± 0.03^{b}	0.36 ± 0.00^{b}	2.27 ± 0.00^{cd}	0.19 ± 0.00^{d}	0.72 ± 0.01^{f}	7.82 ± 0.13^{cd}	46.58 ± 0.34^{e}	275.50 ± 6.06^{ef}

Different letters within the same column are different at $p<0.05$

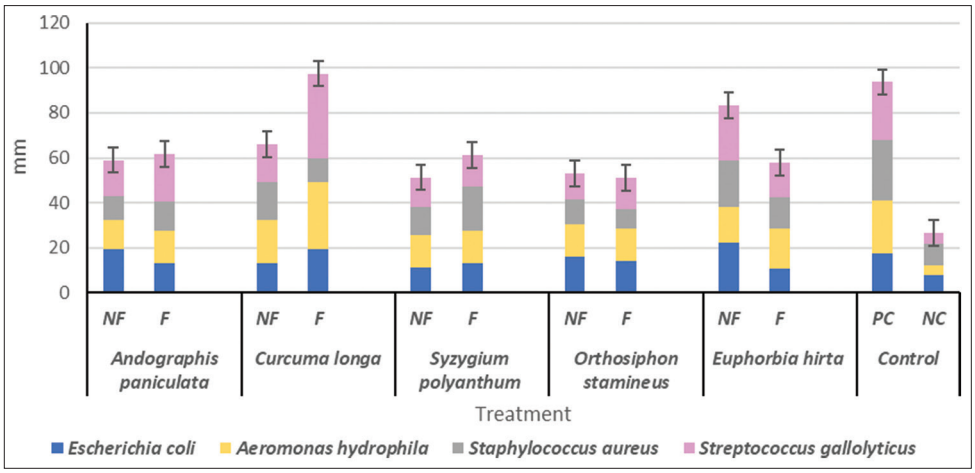

Figure 1: Pathogenic bacteria susceptibility to fermented and non-fermented herbs extracts (NF=Non fermented, F=Fermented, PC=Positive control, NC=Negative control)

The extracts of *O. stamineus* exhibited inhibitory effects, with the fermented extract showing a slightly reduced impact compared to the non-fermented extract. The fermented extract of *E. hirta* showed reduced antibacterial activity compared to the non-fermented extract.

It was discovered that all herbs, regardless of whether they were fermented or not, exhibited greater antibacterial activity compared to the negative control. However, their activity was not as potent as the positive control, which was tetracycline. Among the several herbs tested, only *C. longa* exhibited antibacterial efficacy comparable to that of tetracycline. Hence, the process of fermentation boosts the antibacterial characteristics of *C. longa* against harmful bacteria, indicating that fermentation could be a helpful technique to enhance the effectiveness of *C. longa* as an antimicrobial agent. This finding also suggests the possible application of fermented *C. longa* in the development of more strong antibacterial therapies (Jyotirmayee & Mahalik, 2022).

Yong *et al.* (2019) found that fermentation increases the concentration of curcumin in turmeric without causing any increase in its toxicity to cells. This process also enhances the medicinal properties of turmeric, suggesting that it could be used as a functional food ingredient. Syawal *et al.* (2021) demonstrated that the secondary metabolites derived from fermented *C. longa*, comprising flavonoids, vitamin C, essential oils, tannins, and curcuminoids, enhance the immune system and haematological and physiological characteristics of catfish.

CONCLUSION

The fermentation of herbs using *S. cerevisiae* significantly enhances their nutritional value and antibacterial properties, making them viable alternatives to antibiotics in animal feed. The notable increase in protein content and the effective antibacterial activity against pathogenic bacteria, particularly with fermented *C. longa*, underscores the potential of these fermented herbs to improve poultry health and meet consumer demands for antibiotic-free poultry products. Future research should focus on optimizing fermentation techniques and understanding the mechanisms behind these beneficial effects to further advance the application of fermented herbs in sustainable poultry farming.

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