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Potential of Bioactive Compounds in Four Accessions of Marigold (*Tagetes Erecta* L.) Based on Gc-Ms Analysis of Poultry Feed Additives

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A B S T R A C T

The use of synthetic feed additives, especially growth-promoting antibiotics, in the poultry industry raises several issues, including microbial resistance, residues in animal products, and health and environmental impacts. Therefore, the development of plant-based feed additives is a more sustainable option. Marigold flowers (*Tagetes erecta* L.) are reported to be rich in bioactive compounds. However, studies on variation in their chemical profiles and their potential as poultry feed additives remain limited. This study aims to analyze the bioactive chemical compounds of four marigold accessions (WRN6, WRSJ, WRSI2, and WRDP) and assess their potential as feed supplements. Marigold flower extraction was performed by maceration using methanol, followed by analysis of volatile and semi-volatile compounds using gas chromatography-mass spectrometry (GC-MS). Data were analyzed descriptively and comparatively, with multivariate statistical analysis used to support the analyses. The results showed that all accessions contained various bioactive compounds, including functional fatty acids, tocopherols, phytosterols, triterpenoids, phenolic compounds, carotenoids, and sulfur compounds, with variations in composition and proportion between accessions. Accession WRSI2 showed the most significant potential as a functional feed additive, followed by WRN6, WRDP, and WRSJ.In conclusion, selecting marigold access based on bioactive compound profiles can provide a scientific basis for developing natural feed additives for poultry that are safe, effective, and sustainable

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INTRODUCTION

In the modern livestock industry, the use of synthetic feed additives, particularly antibiotics and other chemical compounds, remains common to improve livestock performance and health. However, the use of these additives raises several serious issues, including increased microbial resistance, accumulation of antibiotic residues in animal products, and potential negative impacts on human health and the environment (Saragih et al., 2018). This situation has prompted the need to develop safer, more sustainable, and environmentally friendly feed additive alternatives, particularly in poultry production systems.

Various studies have shown that bioactive compounds derived from plants have great potential as substitutes for synthetic feed additives. These compounds have been reported to inhibit the growth of pathogenic microorganisms, improve feed utilization efficiency, and support the health of the poultry digestive tract (Pasaribu, 2019). Phytobiotics, obtained from plant extracts or specific plant parts, are known to have antimicrobial, antioxidant, and immunomodulatory activities, and to play a role in improving intestinal histomorphology and feed conversion in poultry (Cungihan, 2024). The effectiveness of phytobiotics is primarily related to the content of phytochemicals such as flavonoids, terpenoids, polyphenols, and alkaloids, which help improve the antioxidant status and immune response of livestock.

In Indonesia, various local plants have been extensively explored as sources of natural feed additives, including turmeric, ginger, temulawak, sambiloto, moringa, and betel leaf, which are known to have antibacterial, antioxidant, and anti-inflammatory properties (Djunaidi et al., 2023; Edi, 2020). The use of herbal plants as phytobiotics has also been reported to improve feed digestibility, feed conversion efficiency, and poultry immunity, and to exhibit synergistic effects when used in certain combinations (Sami & Fitriani, 2019). However, further exploration of other plants high in secondary metabolites and with potential as poultry feed additives is needed.

One plant with great potential as a natural feed additive is marigold (Tagetes erecta L.). This plant contains various secondary metabolites, including fatty acids, terpenoids, tocopherols, phytosterols, flavonoids, and carotenoids, which exhibit antioxidant and antimicrobial activities (Edi, 2020). The main carotenoids in marigold, especially lutein and zeaxanthin, have been reported to improve the skin color of broiler chickens and the yellow color of eggs, while reducing oxidative stress in poultry (Abd-Alla, 2022). In addition, the bioactive compounds in marigolds also contribute to improving the immune system and metabolic health of poultry (Paredes & Quispe, 2022).

Although the potential of marigold as a natural feed additive has been reported, studies linking variations in the bioactive compound profile of marigold at the intra-species level, particularly through Gas Chromatography–Mass Spectrometry (GC-MS) analysis, remain limited. In fact, differences in secondary metabolite profiles at the intra-species level can affect their biological effectiveness as phytobiotics in poultry feed.

Therefore, the novelty of this study lies in selecting marigold accessions based on GC-MS analysis of bioactive compound profiles at the intra-species level, a practice still rarely reported in poultry feed additive studies. This approach is not only descriptive but also applicable in supporting the development of poultry phytobiotics as an alternative to antibiotic growth promoters. Thus, this study is expected to contribute, both scientifically and practically, to the development of effective, safe, and sustainable poultry feed additives.

LITERATURE REVIEW

Financial integration within the ASEAN region has intensified over the past two decades, leading to increased cross-border capital flows and greater interconnectedness among national banking systems. According to the Asian Development Bank (ADB, 2019), ASEAN financial integration aims to enhance regional financial stability, efficiency, and competitiveness. However, higher integration also increases exposure to global economic shocks, making banking performance and resilience critical issues for policymakers and market participants.

Banking performance is commonly evaluated using financial ratios that reflect profitability, liquidity, capital strength, efficiency, and credit risk. Return on Assets (ROA) is widely used to measure a bank's ability to generate profit from its total assets and is considered a key indicator of managerial efficiency (Golin & Delhaise, 2013). Studies by Athanasoglou, Brissimis, and Delis (2008) confirm that ROA effectively captures the overall performance of banks across different economic environments.

Liquidity management is often assessed through the Loan to Deposit Ratio (LDR), which reflects a bank's capacity to transform deposits into earning assets. While higher LDR values may indicate aggressive lending strategies, excessively high ratios can increase liquidity risk (Saunders & Cornett, 2018). Research by Vodová (2011) shows that optimal liquidity levels are essential for maintaining financial stability, particularly during periods of economic stress.

Capital Adequacy Ratio (CAR) serves as a key indicator of a bank's financial resilience and ability to absorb losses. Basel III regulations emphasize the importance of strong capital buffers to mitigate systemic risk (Basel Committee on Banking Supervision, 2011). Empirical evidence from Berger and Bouwman (2013) demonstrates that well-capitalized banks are more likely to survive financial crises and maintain stable lending activities.

Operational efficiency is commonly measured using the Operating Expenses to Operating Income ratio (BOPO). Lower BOPO values indicate better cost management and higher operational efficiency. Studies by Berger and Humphrey (1997) suggest that efficient banks are more competitive and better positioned to withstand economic volatility. In contrast, high BOPO ratios often reflect structural inefficiencies and weak cost controls.

Credit risk is typically assessed using the Non-Performing Loans (NPL) ratio. High NPL levels signal deteriorating asset quality and increased default risk, which can undermine banking stability (Louzis, Vouldis, & Metaxas, 2012). Research across ASEAN countries by Karim et al. (2010) finds that macroeconomic conditions and bank-specific management practices significantly influence NPL levels.

Comparative studies of ASEAN banking systems reveal structural differences across countries. Indonesia's banking sector is often characterized by higher profitability but greater volatility, while Malaysia and Singapore exhibit stronger regulatory frameworks and more stable performance (World Bank, 2020). These differences highlight the role of institutional quality, regulatory effectiveness, and risk management strategies in shaping banking outcomes.

Overall, the existing literature underscores that variations in banking performance across ASEAN countries are influenced by both internal management factors and external economic conditions. This study contributes to the literature by providing a comparative analysis of banking performance in Indonesia, Malaysia, and Singapore during the post-pandemic period, offering insights relevant to bank management, regulators, and investors.

Poultry Feed Additives and Synthetic Antibiotics Issues

The use of synthetic feed additives, particularly antibiotic growth promoters (AGPs), has long been used to improve poultry production performance. However, various studies report that this practice contributes to increased antimicrobial resistance, antibiotic residues in livestock products, and potential negative impacts on human health and the environment. These conditions have prompted a paradigm shift toward the development of safer, more sustainable natural feed additives.

Phytobiotics as an Alternative Feed Additive

Phytobiotics are plant-based feed additives that contain bioactive compounds with antimicrobial, antioxidant, and immunomodulatory activities. Various studies show that compounds such as flavonoids, terpenoids, phenols, alkaloids, and carotenoids can improve digestive tract health, feed conversion efficiency, and the immune status of poultry. The mechanism of action of phytobiotics involves inhibiting pathogenic microbes, protecting cells against oxidative stress, and modulating the immune response, making them a potential replacement for AGPs in modern poultry production systems.

The Potential of Marigold as a Feed Additive

Marigold is reported to be one of the plants with high levels of secondary metabolites, including functional fatty acids, terpenoids, tocopherols, phytosterols, phenolic compounds, carotenoids, and sulfur compounds. Major carotenoids such as lutein and zeaxanthin play an important role in improving poultry product quality, particularly skin and egg yolk coloration, and function as powerful antioxidants. In addition, other bioactive compounds in marigolds contribute to antimicrobial activity and improved poultry immunity.

Intra-Species Variation and Research Gap

Although the potential of marigolds as a feed additive has been widely reported, most studies have focused on the species level and have not examined the variation in bioactive compound content at the accession level. In fact, genetic and environmental factors can cause significant differences in the composition and proportions of secondary metabolites among accessions. This limitation indicates a research gap in identifying the most effective and safe marigold accessions as poultry feed additives.

GC-MS Analysis as a Basis for Access Selection

Gas chromatography–mass spectrometry (GC-MS) analysis is a practical analytical approach for identifying and comparing volatile and semi-volatile compounds in plant materials. The use of GC-MS enables comprehensive chemical profiling, which can serve as a scientific basis for selecting marigold accessions based on their bioactive potential. This approach integrates chemical data with functional implications in poultry nutrition.

Novelty and Contribution of Research

Based on a literature synthesis, the novelty of this research lies in selecting marigold accessions based on bioactive compound profiles determined by GC-MS analysis at the intra-species level. This approach fills a gap in previous research. It makes an important contribution to the development of more standardized, effective, and sustainable poultry phytobiotics as an alternative to synthetic feed additives.

METHODS

Type and Design of Research

This research is a quantitative-experimental laboratory study that applies a comparative chemical analysis approach to evaluate variations in bioactive compound profiles among various marigold (Tagetes erecta L.) accessions. The research design focused on mapping, identifying, and comparing the composition of volatile and semi-volatile compounds in each accession, providing a scientific basis for assessing their relative potential as poultry feed additives. This approach emphasizes phytochemical analysis as the basis for phytobiotic development, aiming to obtain marigold accessions with the most supportive bioactive compound composition for the expected biological activity in poultry feed systems (Edi, 2020; Paredes & Quispe, 2022).

Research Population and Samples

The research population comprised all available marigold accessions (*Tagetes erecta* L.) that are suitable for development as natural feed additives. From this population, four marigold accessions—WRN6, WRSJ, WRSI2, and WRDP—were selected as research samples. Sample selection was conducted using a purposive sampling approach, based on considerations of genetic diversity among accessions and their potential influence on the variability of secondary metabolite composition. Such intra-species variation has been reported to significantly affect the content and profile of bioactive compounds in plants, thereby justifying the targeted selection of accessions for comparative phytochemical analysis (Lohar et al., 2018).

Extraction Procedure

Marigold flowers were first air-dried to reduce moisture content, then finely ground to increase the surface area for solvent contact prior to extraction. The powdered samples were subsequently extracted by maceration with methanol. Methanol was selected for its effectiveness in extracting polar and semi-polar bioactive compounds and for its widespread application in phytochemical studies of feed crops and phytobiotics (Edi, 2020). The maceration was conducted at room temperature to preserve thermolabile compounds, after which the extract was filtered, and the filtrate was concentrated by solvent evaporation to obtain a viscous extract suitable for further chemical analysis.

Research Tools and Instruments

The primary analytical instrument used in this study was gas chromatography–mass spectrometry (GC-MS), which was employed to identify and profile volatile and semivolatile compounds in marigold extracts. GC-MS was selected due to its high sensitivity, accuracy, and reliability in separating complex mixtures and providing detailed mass spectral information for compound identification. This technique has been widely applied in the phytochemical characterization of plants used as feed additives, making it particularly suitable for comparative analysis of bioactive compound profiles among marigold accessions (Paredes & Quispe, 2022).

Data Analysis Techniques

The GC-MS analysis generated data on the types of compounds detected and their relative abundance expressed as percentage composition, which were analyzed descriptively and comparatively among marigold accessions. Comparative analysis was conducted to identify variations in chemical profiles between accessions. To strengthen the interpretation of these compositional differences, the dataset was further subjected to multivariate statistical analysis, allowing for an objective evaluation of similarity and divergence patterns among accessions based on their bioactive compound profiles (Lohar et al., 2018). The results of the chemical analysis were subsequently interpreted in relation to existing literature on the biological roles and functional relevance of bioactive compounds in poultry nutrition, particularly their potential contributions to antioxidant capacity, health status, and feed additive functionality (Abd-Alla, 2022; Edi, 2020).

Research Model and Framework

This research model positions marigold accession as the independent variable and the profile of bioactive compounds obtained from GC-MS analysis as an intermediate variable, which subsequently serves as the basis for evaluating marigold's functional potential as a poultry feed additive. The research framework is constructed on the assumption that genetic variation among accessions leads to differences in the composition and relative abundance of secondary metabolites. These variations are expected to influence the biological properties of the extracts, particularly antioxidant, antimicrobial, and immunomodulatory activities relevant to poultry nutrition and health (Paredes & Quispe, 2022).

Hypothesis Testing Techniques

The research hypothesis was examined indirectly through comparative analysis of bioactive compound profiles among marigold accessions, followed by functional interpretation based on relevant literature. Accessions characterized by a dominance of bioactive compounds associated with poultry health and productivity were considered to exhibit greater potential as natural feed additives. This indirect evaluative approach aligns with previous phytobiotic studies that employed phytochemical composition as the primary basis for assessing the biological and functional potential of feed crops in poultry nutrition systems (Edi, 2020; Abd-Alla, 2022)

RESULT

Verification of Research Hypothesis

This research hypothesis posits that there are differences in the profiles of bioactive compounds among marigold accessions, with implications for their functional potential as poultry feed additives. The results of GC-MS analysis of four marigold accessions (WRN6, WRSJ, WRSI2, and WRDP) support this hypothesis, as indicated by variations in the types, composition, and dominance of bioactive compounds in each accession. The identified compounds include antioxidants, functional fatty acids, phytosterols, triterpenoids, phenolic compounds, and bioactive sulfur compounds, which collectively indicate that marigold has the potential to be a multifunctional natural feed additive.

Antioxidant Compounds and Cell Protection

The majority of bioactive compounds identified in all marigold accessions have antioxidant activity. Fatty acids such as hexadecanoic acid and octadecanoic acid play a role in reducing oxidative stress and maintaining the stability of poultry cell membranes (Elewa et al., 2023; Sari & Muslimin, 2023). Additionally, linoleic acid (9,12-octadecadienoic acid) and conjugated linoleic acid esters contribute to antioxidant and anti-inflammatory activity, which is highly relevant in intensive poultry production systems that are susceptible to oxidative stress.

All accessions contain vitamin E and its derivatives (β- and γ-tocopherol), which play an important role in boosting immune response, preventing lipid peroxidation, and maintaining meat and egg quality (Pirman et al., 2020; Wang et al., 2016). Phytosterols such as campesterol, stigmasterol, and sitosterol also help increase endogenous antioxidant status and reduce oxidative stress biomarkers, such as malondialdehyde.

Squalene, detected in all accessions, acts as a natural antioxidant and supports lipid metabolism, while the presence of lutein, the primary carotenoid in marigold flowers, strengthens antioxidant capacity and improves poultry product quality (Wang et al., 2016; Kurniati, 2021).

Functional Fatty Acids as a Source of Energy

All four marigold accessions contain saturated and unsaturated fatty acids, such as palmitic, stearic, oleic, and linoleic acids, and their derivatives, which serve as the primary energy source and regulators of poultry metabolism. Unsaturated fatty acids, particularly oleic and linoleic acids, play a role in cell membrane formation, transport of fat-soluble vitamins, and increased feed conversion efficiency (Wang et al., 2016; Pirman et al., 2020).

In addition to their energy function, fatty acids are also involved in modulating inflammatory responses and hormone synthesis, which cumulatively support poultry growth and production performance (Alagawany et al., 2019). Variations in fatty acid proportions among accessions indicate opportunities to selectively utilize accessions according to poultry physiological needs (Barut et al., 2023; Yang et al., 2025).

Phytosterols and Digestive Health

Phytosterols such as sitosterol and stigmasterol were detected predominantly in WRSI2 and WRDP accessions. These compounds function as immunomodulators by strengthening cell membrane integrity, lowering cholesterol levels, and reducing inflammation in the digestive tract of poultry (Paredes & Quispe, 2022). The impact is an increase in nutrient absorption efficiency and overall intestinal health. The presence of phytosterols is also associated with increased resistance of poultry to pathogens, thereby supporting the innate and adaptive immune systems.

The Role of Bioactive Triterpenoids

Triterpenoids such as β-amyrin, α-amyrin, and β-amyrone are most abundant in the WRSI2 accession. These compounds have mild anti-inflammatory and antimicrobial activities and play a role in maintaining intestinal mucosal integrity and microbiota balance (Leskovec et al., 2017; Zhen et al., 2021). The anti-inflammatory effects of triterpenoids contribute to a reduction in intestinal damage caused by environmental stress and infection, thereby optimizing nutrient allocation for poultry growth and production (Pirman et al., 2020; Urban et al., 2024).

Table 1. Compounds Identified in Marigold Extract

Compound	WRN6 (Smooth Yellow)	WRSJ (Smooth Cream)	WRSI2 (Maharani Yellow)	WRDP (Smooth Yellow)
+/-Tetrahydro-3-furanmethanol		3.06		
n-Hexadecanoic acid			5.37	
9,12-Octadecadienoic acid (Z,Z)-		23.58	10.63	30.72
Octadecanoic acid	8.64			7.38
Squalene	4.14	2.71	2.76	1.99
Hexadecane, 1-iodo-	2.38	1.71	3.53	
beta-Tocopherol			2.52	
gamma-Tocopherol			1.43	2.05
Gamma-Tecoperol	1.10			
Hentriacontane	2.30	1.14		
Vitamin E	7.46		4.43	11.35
gamma-Sitosterol	2.95	3.54	5.46	
beta-Amyrin	11.06	5.73	23.82	3.22
alpha-Amyrin	2.54	2.11	4.27	
Benzoic acid, 4-hydroxy-3,5-dimethoxy-	4.77	3.36		
Hexadecanoic acid, methyl ester				1.17
n-Hexadecanoic acid	10.21	9.87	5.37	12.46
2,2′;5′,2′′-Terthiophene	5.82			
Eicosane			1.45	
Tetradecane	2.49			
Linoleylmethyl ketone				3.69
Octacosane	1.20		1.95	

Compound	WRN6 (Smooth Yellow)	WRSJ (Smooth Cream)	WRSI2 (Maharani Yellow)	WRDP (Smooth Yellow)
Pyridine-3-carboxamide, oxie, N-(2-trifluoromethylphenyl)-	3.43		1.13	
Linoelaidic acid		2.43		
Nonadecadane, 9-methyl-		3.41		
Stigmasterol			4.24	
9-Octadecenoic acid, methyl ester, (E)-				1.38
Cis-Vaccenic acid				2.67
Heptadecane				1.34
Beta-Amyrone				1.71

Phenolic Compounds and Antimicrobial Activity

Phenolic compounds and their derivatives, such as substituted phenols and oxygenated benzoic acids, have potential as natural antimicrobials through mechanisms of cell membrane disruption and inhibition of microbial enzyme activity (Diarra et al., 2019; Urban et al., 2024). In addition to antimicrobial activity, phenolic compounds also have antioxidant properties that support cell health and the immune system of poultry (Abd-Alla, 2022).

Bioactive Sulfur Compounds

The bioactive sulfur compound 2,2';5',2''-terthiophene was identified explicitly in accession WRN6. This compound has antimicrobial and antiparasitic activities that have the potential to reduce the incidence of infectious diseases in poultry (Abdelli et al., 2021). The synergy between sulfur compounds and other phytobiotics enhances the protective effect on poultry health (Mnisi et al., 2022; Oni & Oke, 2025).

Mapping of Functional Potential Among Accessions

Based on the synthesis of results, the WRSI2 accession was classified as the most superior functional feed additive due to its dominance in antioxidants, triterpenoids, and functional fatty acids. The WRN6 accession stood out as a natural antioxidant and antimicrobial, WRDP excelled in supporting energy metabolism and feed efficiency, while WRSJ acted as a supplement with moderate bioactive activity. This grouping is relevant to the needs of modern poultry production systems that aim to reduce the use of synthetic antibiotics and develop sustainable phytogenic feed additives (Pasaribu, 2019; Ilham et al., 2023).

CONCLUSIONS

GC-MS analysis shows that all marigold accessions (WRN6, WRSJ, WRSI2, and WRDP) contain bioactive compounds with variations in composition that reflect differences in function at the intraspecific level. The WRSI2 accession has the most balanced and functional compound profile among poultry feed additives. At the same time, WRN6 excels as a source of antioxidants and antimicrobials, WRDP has the potential to support energy metabolism and feed efficiency, and WRSJ shows relatively low bioactive activity. These findings confirm that selecting marigold accessions based on their bioactive compound profiles is a strategic scientific approach in developing safe and sustainable poultry phytobiotics as alternatives to synthetic antibiotics.

WRSI2 accession is recommended as a priority for the development of multipurpose poultry feed additives, while WRN6 and WRDP are directed towards specific phytobiotic formulations. Further in vivo research is needed to confirm the effectiveness, optimal dosage, and impact on poultry performance and health to support industrial applications.

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