

Identification of Phenotypic, Nutrition and Bioactive Compounds of Marigold (*Tagetes erecta*) in Bengkulu Province, Indonesia as Poultry Feed Material

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ABSTRACT

Marigolds (*Tagetes erecta*) belong to the widely developed astraceae family and are easy to cultivate. Marigold flowers have a high content of nutrients and active compounds so that the potential to be used as poultry feed. This study aims to identify phenotypes, nutrients and bioactive compounds Marigold found in Bengkulu province Indonesia. The study was conducted using purposive sampling survey method by conducting non-experimental descriptive observations. The variables observed were phenotypic identification, nutrient content and bioactive compounds. There are 7 (seven) Marigold Accessions, Nutritional content, especially kasar protein, is quite good at 8.75-14.23% with the highest content in the wrll accession which is almost the same as WRRM and WRDP while the lowest is WRSJ. While the coarse fiber content is almost the same ranging from 9.19-12.08%. Accession WRRL has the lowest tannin and HCN content of 7.80% and 0.64%. Carotenoids were moderately high (327 mg/kg) at the accession of WRDP. The accession of WRRL has a better content of nutrients and bioactive compounds compared to other Accessions this shows that marigold can be used as an alternative to natural feed additives to increase poultry productivity

INTRODUCTION

Marigold (*Tagetes erecta*) is an annual plant from the *Astraceae* family, reaching a height of 0.5 m to 1.5 m from the ground surface and has taproots and is included in the dicotyledon class. Marigold stems are greenish white when the shoots are young and become completely green when they have reached maturity with an upright and branched growth type. Marigold stems and leaves are covered with fine hairs with dark green leaves, lanceolate in shape, serrated or wavy leaf edges with pointed tips. The length of the leaves ranges from 5 cm to 10 cm and is a compound leaf (Gupta & Neeru, 2012; Singh et al., 2020; & Kurniati, 2021).

Marigolds originate from the American continent, but over time this plant has been widely cultivated throughout the world. Marigolds have many other names in Indonesia, namely kotok tai flower (West Java), gemitir (Bali) and tahi ancok (Sumatera). Marigold plants (*Tagetes erecta*) are widely used as cut flowers in religious ceremonies and offerings in several areas such as Bali. The need for Marigold flowers in Bali reaches an average of 8 tons per day which can increase to 40 tons per day approaching big day celebrations or religious ceremonies which is estimated to reach 100-200 billion rupiah per year. Meanwhile, the need for marigold flowers in Indonesia reaches 40 tons day-1. Therefore, marigold flowers are one of the superior ornamental plants and have high economic value (Kurniati, 2021). The high demand for marigolds and the many benefits from various fields indicate that this commodity has high value for further development. In addition to being used as ornamental plants, marigold flowers can also be used as food coloring in the culinary sector. Andria (2021) reported that the addition of 0.5% marigold dye powder can provide the best chemical, physical and sensory characteristics in making wet noodles. In the agricultural sector, it functions as a biological agent to attract plant pests. Marigold is generally used by the community as a fungicide, insecticide, and anti-nematode because it contains terpenoids. In addition to containing terpenoids, marigolds also contain carotenoids.

Marigold flowers contain 0.1-0.2% carotenoids in dry matter with 80% of them being lutein (Sivel, Fisera & Kuban. 2014). Until now, marigold flower crowns have become one of the main sources for lutein production (Pajaree et al. 2015). Lutein acts as an antioxidant that can reduce free radicals and reactive molecules so that it can inhibit cell damage (Winarsih. 2007).

One of the ingredients rich in carotenoids is marigold flowers which are widely used as a source of natural carotenoids in poultry (Britton et al., 2001). Marigold flower extract contains 27% carotenoid pigments and specifically for the crown is about 200 times greater than the carotenoids contained in yellow corn (Seafast Center. 2012). Uly, et al (2017) reported that marigold flowers contain 119.22 mg/g of carotenoids. Beta-carotene (provitamin A contained in marigold can be converted into vitamin A in the body of poultry. The function of carotene is not only as a dye, but also for the growth and reproduction of livestock (Nuraini, Mirzah & Djulardi. 2017). The addition of marigold in chicken feed can intensify the color of egg yolk and broiler chicken meat (Navarrete, et al., 2005). Xanthophyll is an oxidized carotenoid derivative and is

used as a feed additive in feed to help form color pigments. Marigold is a natural source of xanthopyll which is used as a natural feed additive to brighten egg yolk and poultry skin. Marigold is also effective for use as a fabric dye, where flower extract contains ethanol which can produce different colors on fabric (Shaifullah., et al, 2018).

In Indonesia, marigold flowers have long been known, but have not been developed on a large scale and are not superior flowers like chrysanthemums, jasmine, orchids, roses and others. The center of marigold plants in Indonesia is located in Bali Province such as Petang District, Baturiti District, Badung Regency, Tabanan Regency and Kintamani District (Nata, et al., 2020). Bengkulu Province has a varying altitude ranging from 0-1000 meters above sea level (masl), has quite high rainfall, an average of 100 mm/year, with an average temperature varying from 24°C-32°C (BMKG, 2024). These conditions are very suitable and support the growth and development of marigold flower plants.

Marigold flowers have many benefits because they contain several compounds that act as antioxidants, including flavonoids, phenolics and crotonoids (Santi., 2021). However, research on the nutritional content and bioactive compounds of marigold flowers is still very limited, especially local varieties in Bengkulu Province. Therefore, it is necessary to conduct a study of the analysis of the nutritional content and bioactive compounds of marigold flowers found in Bengkulu Province. This study aims to determine the phenotypic identification, nutritional content and bioactive compounds of marigold flowers found in Bengkulu Province.

METHODS

Morphological Identification

Phenotypic identification was carried out by non-experimental descriptive observation through identification of phenotypic characters using a survey method with purposive sampling. Determination of data sampling locations was based on a preliminary survey. The survey location was in Bengkulu Province covering 7 districts/cities, namely Bengkulu City, Kepahiyang Regency, Rejang Lebong Regency, Central Bengkulu, Seluma, South Bengkulu and Kaur Regency. Samples were observed and measured directly including flower diameter, flower weight, flower color, crown shape, leaf shape, and stem shape.

Preparation of Marigold Flower Accession

The harvested marigold flowers are then separated from the stems and petals. After that, drying is carried out using a sanggai machine at a temperature of 70°C for 24 hours. The dried marigold flower crowns are then ground to obtain marigold flower powder (*Tagetes erecta*).

Nutritional Component Analysis

The nutritional content (dry matter, ash, minerals, crude fat, crude protein and crude fiber) of marigold flower powder was determined according to the Association of Official Analytical Chemists (AOAC) method (2005).

Bioactive Compounds Analysis

The bioactive compounds of marigold flowers measured in this study is the content of carotenoids, HCN and tannins.

Carotenoid content was measured using a Spectrophotometer based on the method carried out by Hendry & Grime (1993). The flower sample was weighed 0.5 g and ground then extracted with 50 ml of 80% acetone solution. The extract was filtered with filter paper and clarified in a centrifuge. The filtrate (filtered result) of 20 ml was put into a spectrophotometer cuvette tube. Furthermore, the filtrate was measured for absorbance at wavelengths of 480 nm, 645 nm, and 663 nm. Carotenoid content ($\mu\text{mol/g}$) was calculated using the equation:

$$\text{Carotenoid} = (A_{480} + 0,114 + A_{663} - 0,638 \times A_{645}) \times V \times 10^3 / 112,5 \times W$$

Description:

A_{480} = Absorbance at a wavelength of 480 nm

A_{645} = Absorbance at a wavelength of 645 nm

A_{663} = Absorbance at a wavelength of 663 nm

V = Filtrate volume (ml)

W = Weight (g)

Analysis of HCN

Levels (AOAC, 1984) A total of ± 10 g of sample was weighed into a kjeldahl flask, add 100 ml of distilled water, macerate for 2 hours. Add another 100 ml of distilled water. The distillate was collected in an Erlenmeyer flask filled with 20 ml of 2.5% NaOH up to 150 ml. Add 8 ml of 14 molar NH_4OH , 5 ml of 5% KI. Titrate with 0.02 N AgNO_3 until turbidity occurs (Sudarmadji *et al.*, 1994). To determine the HCN level in a sample, the formula can be used::

$$1 \text{ ml AgNO}_3 = 0,54 \text{ mg HCN (ppm)} = \frac{\text{ml titrasi AgNO}_3}{\text{mg sample}} = 10^6$$

Description : $10^6 = 10 \times 10 \times 10 \times 10 \times 10 \times 10 = 1.000.000$

Tannin content analysis was carried out using the Makkar method (2003) and calibrated with a standard solution of tannic acid 0.1 mg/ml (Merck). Total tannins were measured using the Makkar method (2003) modified by using Folin-Ciocalteu using polyvinyl polypyrrolidone (PVPP) to separate tannin phenol from NTP. The sample was emitted with a UV-Vis spectrophotometer at a wavelength of 724 nm.

Data analysis

Data from phenotypic identification, nutritional analysis and bioactive compound analysis were analyzed descriptively.

RESULTS

Steps of Your Result Test Here

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Figure 1. Survey Locations of Marigold (*Tagetes Erecta*) Accessions in Bengkulu Province, Indonesia. 1. Bengkulu City; 2. Kepahiang; 3. Rejang Lebong; 4. Central Bengkulu; 5. Seluma, 6. South Bengkulu; 7. Kaur

Phenotypic characterization shows that marigold stems have a sympodial branching type (new growth occurs from branches), upright stems and are round in shape. Leaves are the main organs that support plant growth and development by providing food processed through photosynthesis. Leaf size directly affects the photosynthesis process and products. According to observations, young marigold flower leaves are lighter green than marigold flower leaves that have reproduced. The length of the leaves ranges from 5 cm to 10 cm and is a compound leaf (Gupta & Neeru, 2012; Singh et al., 2020; and Kurniati, 2021).

Based on the proportion of leaf size, it is generally elongated or oblong. The leaves are alternately opposite, the leaf blades are lanceolate (the widest part of the leaf is in the middle of the leaf blade), the arrangement of the leaf veins is pinnate, the leaf tips are pointed, the leaf vein branches reach the edge of the leaf and the leaf surface is coarsely hairy. Marigold flowers have a diameter of 32.94-37.33 mm with a flower weight of 4.07-6.69 g as presented in Table 1.



Figure 2. Marigold (*Tagetes Erecta*) Leaves and Stems

Marigold plants are annual herbs (*annual plants*) with a height of 0.5 m to 1.5 m from the ground surface and have taproots and are included in the dicotyledon class. Marigold stems are greenish white when the shoots are still young and become green overall when they have reached maturity with an upright and branched growth type. Marigold stems and leaves are covered with fine hairs with dark green leaves, lanceolate in shape, serrated or wavy leaf edges with pointed tips. The length of the leaves ranges from 5 cm to 10 cm and is a compound leaf (Gupta & Neeru, 2012; Singh *et al.*, 2020; and Kurniati, 2021).

Marigold has flowers with bright colors, namely white, yellow, orange, golden yellow, or double colors influenced by carotenoid pigments with the appearance of flowers resembling pompons or round and many. The results of the study found 7 marigold accessions (*Tagetes erecta*) with different phenotypic appearances as presented in Figure 3.



Figure 3. Marigold (*Tagetes Erecta*) Accessions Found in Bengkulu Province

Seven marigold accessions found in Bengkulu Province have various colors ranging from cream to bright orange. The results showed that the diameter of marigold flowers ranged from 32.94-37.33 mm with the widest crown diameter of accession WRRL (37.33 mm) and the smallest WRSJ (32.94 mm). The diameter of this marigold flower is smaller than that reported by Kurniati (2021). The flower diameter ranges from 7 cm to 10 cm, in the form of a tuber, single or collected in a panicle, with a double flower crown. Marigold flowers also have a pungent aroma so that they have the potential to be used as

repellent plants in the concept of integrated pest control (IPM) (Kurniati, 2021). The phenotypic differences of marigold flowers in Bengkulu Province are presented in Figures 2 and 3.

Table 1. Phenotypic Characterization of Various Marigold (*Tagetes erecta*) Flower Accessions in Bengkulu Province

Accession	Flower diameter (mm)	Weight flower (g)	Color	Petals	Leaf	Rod shaped
WRRL	37.33	6.69	Orange	Symmetrical (small size petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color
WRLB2	34.80	4.86	Orange	Asymmetrical (The size of upper petal is smaller than the bottom petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color
WRRM	34.71	4.90	Orange	Symmetrical (big size petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color
WRDP	34.26	4.95	Yellow	Symmetrical (small size petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color
WRN6	32.94	4.07	Yellow	Asymmetrical (The size of upper petal is smaller than	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching

Accession	Flower diameter (mm)	Weight flower (g)	Color	Petals	Leaf	Rod shaped
				the bottom petal)		g and green in color
WRLB1	35.95	4.36	Cream	Symmetrical (big size petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color
WRSJ	36.03	6.35	Cream	Symmetrical (small size petal)	Pinnate compound leaves (pinnatus)	Round stem, sympodial branching and green in color

Nutritional Component Analysis

The results showed that the mineral content was 5.62-8.19% as presented in Table 2. Minerals are one of the feed substances that are relatively little needed but have an important role in the growth process of poultry. Minerals are one of the nutrients that must be met in poultry feed. Mineral deficiency can cause decomposition of chicken organs such as bones. The crude fat content has an average that is almost the same in each accession, namely 1.41-4.48%. Crude fat in poultry is used for egg production, fat in meat and as a reserve source of energy for poultry activities. The addition of fat in broiler chicken rations can increase energy content, while fat deficiency will inhibit the growth of broiler chickens (Suprijatna *et al.*, 2005).

The crude protein content of a feed ingredient is influenced by variety factors, planting area and cultivation factors (Lachman *et al.*, 2005). Plants require more nitrogen food in vegetative growth therefore plants efficiently store protein at the early stages of growth that is consumed, then during the flowering and fruiting period followed by a dormant phase while the nutrient content decreases (Ahmed, 2013). The development of different plant genotypes produces different protein content (Deans, *et al.* 2016). The WRRL accession with a small orange crown has the highest crude protein content of 13.83% which is almost the same as the protein content of the WRRM orange crown with a wide shape and the lowest protein in the WRSJ accession with a cream-colored crown. Protein is one of the nutritional factors in the growth of broiler

chickens (Kurniawan *et al.*, 2012). Protein is the main component of biologically active compounds that function to build new tissue and repair damaged or shrunken tissue. Lack of protein in poultry rations can cause increased fat deposition in the body. This happens because excess energy in the body is not used for growth, so it is stored in the form of fat. Poultry cannot produce protein in their bodies so it must come from feed that is rich in protein content. The results showed that marigold flowers accession WRRL has a higher protein content compared to orange marigold flowers found in Kepahiang Regency is 10.51% (Rita, *et al.*, 2024).

The results of the study showed that the crude fiber content in 7 (seven) marigold accessions was almost the same, namely 9.68-12.08% (as presented in Table 2). Crude fiber in poultry feed plays a role in helping peristalsis to stimulate the development of digestive organs, and also high crude fiber causes poultry to feel full (Londok & Rompis, 2019). High crude fiber will have a negative effect on the digestion and absorption of poultry feed. Ahmed *et al.*, (2013) reported that mature plants usually contain higher crude fiber than young plants. Seasonal variations also affect crude fiber content. As the age of the plant increases, the crude fiber content tends to increase for all plants, but for some plants the increase is higher than other plants. The maturity stage of forage species can also be used to predict fiber content because the fiber content in forage species increases with the maturity stage (Lu *et al.*, 2005). The results showed that marigold flowers accession WRRL has a lower crude fiber content compared to orange marigold flowers found in Kepahiang Regency is 13.34% (Rita, *et al.*, 2024).

Table 2. Results of Nutritional Content Analysis of Various Marigold Flower Accessions (*Tagetes erecta*) in Bengkulu Province

Accession	Dry Material (%)	Ash (%)	Mineral (%)	Crude Fat (%)	Crude Protein (%)	Crude Fiber (%)
WRRL	85.78	5.12	6.82	2.28	14.23	9.68
WRLB2	86.75	7.27	7.17	4.34	12.19	12.08
WRRM	75.05	6.36	8.19	3.73	13.83	9.32
WRDP	85.55	5.31	7.25	4.88	13.81	10.23
WRN6	86.96	4.83	5.62	1.79	10.08	9.64
WRLB1	83.69	5.95	6.46	3.87	13.27	9.66
WRSJ	85.48	4.97	5.73	1.41	8.75	9.19

Carotenoid

The highest carotenoid content of the accessions found in Bengkulu Province was in marigold flowers with orange crowns with an average carotenoid content of 205,776 mg/kg, while the lowest carotenoid content was in marigold flowers with cream-colored crowns, namely 69.78 mg/kg as presented in Table 3. According to S. D. Carvalho & Folta (2016) reported that light is one of the important factors in the process of photosynthesis so that

sufficient light is crucial in the growth and development of plants. Light intensity also plays an important role in the formation of high amounts of carotenoids (Kopsell *et al.*, 2012). At high light intensities, metabolism or synthesis of carotenoids and other pigment compounds also occurs to prevent damage caused by excess energy that cannot be absorbed by chlorophyll. Basically, the production of the carotene group functions as an assistant pigment that helps absorb excess energy that cannot be absorbed by the photosynthetic organs.

Carotenoids can function as antioxidants to ward off free radicals. The carotenoid commonly found in *Tagetes erecta* is lutein. Lutein is a xanthophyll or oxycarotenoid containing 2 bonds at the end, namely (one beta ring and one alpha-ionone ring) and the main structure of C-40 isoprenoid in all carotenes (Gopi *et al.*, (2012). Xanthophyll compounds with their main components in the form of lutein and zeaxanthin in yellow carotenoids can produce yellow and orange colors in flowers. In order to overcome the problem of high concentrate prices, farmers can utilize available feed but need to add supplements that can meet the nutritional needs of broilers, including *Tagetes erecta* or also known as Marigold has flowers that are rich in nutrients and carotenoids. The nutrients contained in flowers include crude protein, crude fat, crude fiber, BETN and minerals. Carotenoids can function as antioxidants to ward off free radicals. The results of research by Agiang, *et al.* (2011) showed that the use of *Tagetes erecta* flowers in broiler rations can increase body weight and increase antibodies in New Castle Disease and Avian Influenza. In addition, the research results of Susanti *et al.* (2018) also showed that *Tagetes erecta* acts as a natural synthetic pigment that can produce Broilers with good performance, meat, skin, legs and beak colors that are not pale.

In the field of animal husbandry, the use of *Tagetes erecta* in feed can improve poultry production performance. In broiler chickens, *Tagetes erecta* can increase live weight, oxidative status and reduce symptoms of inflammation. In addition, it can also increase antibodies in New Castle Disease and Avian Influenza (Rajput *et al.*, 2012). In laying hens, the use of *Tagetes erecta* extract can give a yellow color to the meat, skin, and feet of broiler chickens. *Tagetes erecta* can also improve feed palatability, hatchability and feed efficiency (Sujatha *et al.*, 2015).

The results of the study showed that the lowest HCN content was in the WRRL accession, which was 0.64 ppm as presented in Table 3. HCN (Cyanide Acid) is an anti-nutritional substance that has a negative effect if it exceeds the optimal limit in chicken rations. The effects of cyanide toxicity on livestock vary and are influenced by several factors, namely: 1) the size and type of animal, 2) the speed at which the animal chews feed, 3) the type of cyanogen in the plant, 4) the activity of enzymes in breaking down feed, and 5) the detoxification power of cyanide. Poultry are more susceptible to HCN poisoning so the maximum limit is lower, namely 10 mg/kg of feed (Jayanegara, Ridla & Laconi, 2019).

Tannins are secondary phenolic compounds of plants found in about 80% of perennial woody plants and 15% of perennial herbs (Mueller Harvey, 1999). Tannins are found in all parts of plants from seeds, fruits, leaves, wood, bark and roots where their main function is to provide protection against microbes, pathogens, insects, pests and herbivores (Dixon *et al.*, 2005). The results of the study showed that the tannin content of 7 marigold accessions was almost the same (7.8-10.02%) with WRRL as the lowest tannin content accession and the highest accession WRLB2. Tannins are anti-nutrients that have the ability to bind proteins. Tannin compounds have an effect on poultry because they inhibit digestion and absorption of nutrients. According to Widodo (2005) that tannin content inhibits poultry growth because tannins can suppress nitrogen and reduce the digestibility of amino acids absorbed by the intestinal villi to be used for the growth and development of body tissues. Tannin content causes the formation of complex compounds with peptide bonds derived from protein, so that it cannot be dissolved in the digestive tract and excreted with feces which will affect the availability of protein. Tannins can affect the nutritional value of feed products by binding minerals and reducing mineral absorption and forming complex compounds with proteins, thereby inhibiting digestion and absorption (Olawoye & Gbadamosi, 2017). The tolerance limit of HCN in chickens ranges from 0.5-3 mg/kg body weight (Hidayat, 2009).

Table 3. Results of Analysis of Bioactive Compound Content (Carotenoids, HCN and Tannins) of Various Marigold Flower Accessions (*Tagetes erecta*) in Bengkulu Province

Acsession	Carotenoid (mg/kg)	HCN (ppm)	Tanin (%)
WRRL	246.93	0.64	7.80
WRLB2	139.57	1.41	10.02
WRRM	123.46	0.87	9.90
WRDP	327.45	0.92	9.29
WRN6	177.14	1.60	9.85
WRLB1	112.73	1.23	9.99
WRSJ	69.78	1.59	9.91

CONCLUSIONS AND RECOMMENDATIONS

There are 7 marigold (*Tagetes erecta*) accessions in Bengkulu Province with phenotypic differences in flower crowns, namely orange and small crowns (WRRL), orange crowns with a small upper crown size while the lower part is large (WRLB2), orange and large crowns (WRRM), yellow and small crowns (WRDP), yellow crowns with a small upper crown size while the lower part is large (WRN6), yellow and large crowns (WRLB1) and cream and small crowns (WRSJ).

The nutritional content, especially protein, is quite good at 8.75-14.23% with the highest content in the WRRL accession and the lowest in WRSJ. While the crude fiber content is almost the same from 7 accessions ranging from 8.68-12.08%. The lowest tannin and HCN content is found in the WRRL accession.

Tannin and HCN contained in marigold flowers are still within the recommended limits for poultry feed. The fairly high carotenoids (246.93 mg/kg) in marigolds indicate that marigolds can be used as an alternative natural feed additive to increase poultry productivity. Identify marigolds that contain the best nutrients and phytochemicals as feed additives for poultry..

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FURTHER STUDY

This study still has limitations so that further research is needed related to the topic of Identification of Phenotypic, Nutrition and Bioactive Compounds of Marigold (*Tagetes erecta*) as Poultry Feed Material in order to perfect this study and increase insight for readers.

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