Determination of nutrient content, β-carotene, and antioxidant activity of *Moringa oleifera* extraction using organic solution

Ucop Haroen1, Syafwan2, Kiki Kurniawan2, Agus Budiansyah2

1Faculty of Animal Science, Jambi University, Jambi, Indonesia
2Research Center for Vaccine and Drugs Development, National Research and Innovation Agency, Jl. Raya Jakarta-Bogor Km 46, Cibinong, Indonesia

**ABSTRACT**

**Objective:** The research was conducted to determine β-carotene and antioxidant activities and screening of phytochemical substances of *Moringa oleifera* extraction using organic solution.

**Materials and Methods:** 550 gm of *M. oleifera* leaf flour was macerated. This research was conducted by laboratory experiments using the maceration method. The extraction was performed using three kinds of solvents, which are n-hexane, ethyl acetate, and methanol; for 3 x 24 h, they were concentrated with a rotary evaporator. Then, the flavonoid, phenolic, β-carotene isolation, and antioxidant tests were conducted using the 2,2-diphenyl-1-picrylhydrazyl on each fraction (n-hexane, ethyl acetate, and methanol).

**Results:** The results of weighing each concentrated extract from the maceration process of each fraction (n-hexane, ethyl acetate, and methanol) were 12.67, 35.67, and 49.29 gm, with the total phenolic content (1.4595 ± 0.361, 46.5489 ± 1.832, and 39.74574 ± 0.786) and total flavonoid content of each fraction (3.3056 ± 0.039, 58.6389 ± 2.051, and 48.9056 ± 0.0809), respectively. The antioxidant activity test on the crude extract from the ethyl acetate fractionation showed that the IC50 value was 30.309 mg/ml. The ethyl acetate fraction has a high total phenolic and flavonoid content. The results of the isolation of β-carotene from *M. oleifera* leaf flour were 0.4798 gm, or equivalent to 0.956% carotenoids.

**Conclusions:** Based on the results of the research, *M. oleifera* leaves are identified to have a fairly high antioxidant activity, which is 30.309 mg/ml, resulting from the potential compounds in *M. oleifera* leaves that function as inhibitors of antioxidant activity, which are the groups of phenolic and flavonoid compounds.

**Introduction**

Indonesia is a tropical country with high- and low-level plants rich in biodiversity. It is estimated that 17% of all species on the earth's surface are found in Indonesia [1]. The Indonesian nation has utilized these rich natural resources as sources of food, food coloring, clothing, cosmetics, and medicines [2,3]. Indonesia has a reasonably high ecosystem diversity, and 47 types of natural ecosystems range from areas covered by ice sheets, lowlands, tropical forests, coral reefs, mangrove forests, and savanna ecosystems. In addition, 30,000 of the 250,000 tall plant species are found in Indonesia. Most of these tropical plants function as traditional medicinal and fodder ingredients, but the use of plants as fodder ingredients has not been explored optimally. One attractive plant in terms of phytochemicals, out of the other thousand species, is *Moringa oleifera*, which is one plant that quickly grows in tropical and subtropical regions, such as Indonesia, and is internationally well known as a nutritious plant.

The leaves of *M. oleifera* are in the shape of an oval to obovate with flat leaflets and small size, and they are compound leaves on one stem. Young *M. oleifera* leaves are usually light green and change to dark green as they age. Young *M. oleifera* leaves have a soft texture and the old ones have a slightly stiff and rigid texture. Young *M. oleifera* leaves have a slight bitter taste but are edible and nontoxic [4].

**Correspondence** Ucop Haroen ucop_haroen@unja.ac.id Faculty of Animal Husbandry, Jambi University, Jambi, Indonesia.

**How to cite:** Haroen U, Syafwan, Kurniawan K, Budiansyah A. Determination of nutrient content, β-carotene, and antioxidant activity of *Moringa oleifera* extraction using organic solution. J Adv Vet Anim Res 2022; 9(2):246–254.

http://bdvets.org/javar/
Sample preparation

Moringa oleifera leaves were washed and air-dried for 5–10 days. Then, the dried M. oleifera leaves were blended and sifted to form a powder. The dried powder (550 gm) was macerated using solvents, n-hexane, ethyl acetate, and methanol in a row for 3 × 24 h; and was concentrated with a rotary evaporator to obtain the fractions of n-hexane, ethyl acetate, and methanol [11].

Testing the total flavonoid, total phenolic, and DPPH content

The research method was a laboratory experiment using a sample of M. oleifera powder to test total flavonoids, total phenolics, and antioxidants using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method on each fraction (n-hexane, ethyl acetate, and methanol).

Analysis of total phenolic

Total phenol analysis was determined using the Folin–Ciocalteu method [12]. The sample was extracted with 5 ml of methanol (85%), homogenized, and centrifuged at 3,000 rpm for 15 min to obtain the supernatant. The supernatant was separated from the filtrate by a filtering method to obtain a filtrate. Then, 400 µl of the filtrate was taken by pipette and put into a test tube, and then it was added to the Folin–Ciocalteu solution, which was vortexed until homogeneous. Next, it was let to stand for 6 min, and then 4.2 ml of Na₂CO₃ 5% solution was added. After that, the sample was let to stand at room temperature for 90 min before measuring its absorbance using a UV-Vis spectrophotometer at a wavelength of 760 nm. Standard curves were made by dissolving gallic acid with various concentrations of 5–40 µg/ml. The total phenolic value was calculated using the following regression formula: y = ax + b.

Analysis of total flavonoid

The sample was dissolved using methanol and iron powder (Fe), then HCl2 M was added so that it gave a red color to the solution, indicating a positive flavonoid. Then, the absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 415 nm. We used a quercetin solution with a concentration variation of 0–40 µg/ml. The total flavonoid value was calculated using the following regression formula: y = ax + b.

Antioxidant test analysis (DPPH)

The measurement of antioxidant activity of each extract against DPPH free radicals was based on the method of Yen and Chen [13]. The mixture of a solution of 0.5 ml of DPPH (1 mM in methanol) and the extract solution (10–200 µg

Materials and Methods

Ethical statement

Moringa leaf samples (M. oleifera) were taken from the M. oleifera plant growing in Jambi Province, and as much as 550 gm had been dried. Phytochemical profile testing of M. oleifera leaf samples was carried out at the Natural Organic Chemistry Laboratory of the Indonesia Institute of Science (LIPI), certified by the National Accreditation Committee, No: LP-767-IDN, Tahun 2017 (http://www.kan.or.id/index.php/2-other/180-direktori-klien-lab-penguji-12).

The tools used were distillation apparatus, rotary evaporator Heidolph WB 2000, UV-1700 series spectrophotometer, an electric water bath, oven, UV lamp at 254 nm and 365 nm, Kjeldahl flask, Kjeldahl tube, a set of distillation apparatus, biuret, TLC plate, aluminum foil, filter paper, and the glassware commonly used in laboratories.

The materials used were the samples of M. oleifera leaves, methanol (Merck), ethyl acetate (Merck), n-hexane (Merck), chloroform (Merck), iron (III) chloride (Merck), acetic anhydride (Fission), ammonia (Merck), concentrated sulfuric acid (Merck), acetic anhydride (Merck), crystalline iodine (Merck), and silica gel 60 F₂₅₄ (Merck).
in 2 ml of methanol) was shaken and stood at room temperature for 30 min to provide the optimum reaction time between DPPH and hydrogen atoms donated by the antioxidants. A wavelength of 515 nm was used to measure the resulting absorption. The difference in the amount of absorption between the blank and the sample was used to figure out the percentage of inhibition for the sample.

The damping effect of DPPH scavenging (%) = 
\[1-(A \_0/A_s) \times 100\]

where \(A_0\) = blank absorbent and \(A_s\) = sample absorbent. The percentage of DPPH attenuation activity was plotted against the sample concentration. The attenuation value of 50% (IC\(_{50}\)) was calculated from the graph of the attenuation percentage to the sample concentration. The test was repeated twice; quercetin was used as a comparison. The reducing energy of \(M.\) oleifera leaf extract used for the test: 4 mg of the sample was weighed in a sample bottle, then dissolved in methanol 4 ml to obtain a mother liquor with a concentration of 1,000 µg/ml. Then, a dilution process was carried out as follows: concentration 200 µg/ml, 500 µl pipette of the sample solution; concentration 100 µg/ml, 250 µl pipette of the sample solution; concentration 50 µg/ml, 125 µl pipette of the sample solution; and concentration 10 µg/ml, 25 µl pipette of the sample solution. Each concentration was then filled up to 2.5 ml with methanol.

Isolation and determination of β-carotene content

The isolation and determination of β-carotene content contained in the \(M.\) oleifera leaf samples were modified from standard carotenoid analysis procedures [14]. 50 gm of \(M.\) oleifera leaf powder samples were macerated using methanol for 6 h. Then, the extract was separated from the dregs. The methanol extract was then evaporated with the solvent and washed with 10 ml of saturated NaCl. This saturated solution of methanol and NaCl extract was partitioned with n-hexane solvent as many as 50 times, and a layer of methanol/NaCl separated it. The n-hexane filtrate was saponified with a 5% KOH/methanol mixture for 3 h. After that, the n-hexane layer (top layer) was separated with KOH/methanol precipitation. The n-hexane filtrate was partitioned with n-hexane solvent with the addition of a small amount of distilled water to wash the remaining KOH/methanol layer. Next, it was dried with anhydrous Na\(_2\)SO\(_4\) and air-dried for 24 h. The β-carotene precipitation that forms is then weighed. The AOAC [15] procedure was used to perform a rough analysis of \(M.\) oleifera leaf flour to find out what nutrients it had.

Results and Discussion

The results of the proximate analysis of the nutrient content of \(M.\) oleifera leaf flour can be seen in Table 1. The table shows that the nutrient content of \(M.\) oleifera leaf flour is quite high so that it can be used as an alternative fodder for livestock. In addition, \(M.\) oleifera leaves also contain secondary metabolites, such as phenolic and flavonoid compounds, that act as antioxidants [4]. A simple way to get secondary metabolites out of \(M.\) oleifera plants is to use the maceration method.

The maceration process of \(M.\) oleifera leaf powder with a total of 550 gm uses the extraction method. The maceration process starts with using nonpolar solvents to convert polar solvents. The finely chopped and air-dried samples were extracted by maceration using n-hexane (7 × 2 l), then were macerated with ethyl acetate (8 × 2 l), and finally with methanol (6 × 2 l) for 3 days consecutively. Each extract was concentrated using a rotary evaporator, so the n-hexane, ethyl acetate, and methanol fractions were obtained. Each concentrated extract from the maceration was collected and weighed with a total of 12.67 gm for the n-hexane fraction, which was greenish yellow in color. The ethyl acetate fraction, which was bright red in color, was weighed at 35.67 gm and the dark red methanol fraction was considered at 49.29 gm.

Total phenolic content

The determination of total phenolic content was analyzed using the Folin–Ciocalteu method, which is measured at a wavelength of 770 nm. The phenolic standard used is gallic acid. The absorbance value of the gallic acid standard solution was made in the form of a calibration curve so that a linear regression equation could be obtained, which is as follows: \(y = 0.0477x - 0.0848\), with the correlation coefficient value (R\(^2\)) of 0.9923. The total phenolic content was determined in three fractions, namely the methanol, ethyl acetate, and n-hexane fractions, with the total phenolic content shown in Table 2.

| Nutrient content | Moringa oleifera leaf powder |
|------------------|-----------------------------|
| Dry matter (%)   | 88.93                       |
| Crude protein (%)| 29.45                       |
| Crude fiber (%)  | 8.76                        |
| Crude fat (%)    | 8.41                        |
| Calcium (mg)     | 7.95                        |
| Energy (Kcal/100 gm) | 307.30                  |

Laboratory of Faculty of Animal Science, University of Jambi, 2020.
Based on the fraction that has the highest phenolic content, which is $46.5489 \pm 1.832\%$, it can be concluded that for every 100 gm, the determination results of the total phenolic content of each fraction, the acetate fraction extract contains the phenolic equivalent of $46.5489 \pm 1.832\%$ gallic acid. Compared to the methanol and n-hexane fractions extract, the methanol fraction extract produced $39.74574 \pm 0.786\%$ phenolic compounds, while the n-hexane fraction extract produced $45.95 \pm 0.361\%$ phenolic compounds. Thus, the ethyl acetate fraction is the best solvent to obtain phenolic compounds. Contrary to Kumbhare et al.’s [16] study, the content of phenolic compounds in *M. oleifera* stem bark extracted using methanol as a solvent produced the highest phenolic compounds, namely $50.72\%$ w/w. Kefayati et al. [17] also reported that the methanolic extract of *Euphorbia splendida* Mobayen had the highest total phenolic compounds (TPC) values, $270.74 \pm 0.005$ mg/gm. At the same time, the ethyl acetate fraction extract only produced a TPC of $208.54 \pm 0.010$ mg/gm [18], also reported the extraction of flower moon leaves (*Tithonia diversifolia*) on total phenolic compounds using ethanol as a solvent, producing $1.37\%$ phenolic compounds.

On the contrary, ethyl acetate as a solvent had $1.28\%$ phenolic compounds. Esmeei et al. [19] reported that methanol extract showed the highest total phenolic content in *Trifolium pratense* L. (red clover) plants *in vivo* and *in vitro*, as well as callus tissue compared to ethyl acetate extract. This is because the complex formed by a moiety of phenolic compounds with carbohydrates and proteins can be more easily extracted in methanol than in other solvents.

**Total flavonoid content**

Analysis of the determination of total flavonoid content using standard quercetin for each fraction can be used to determine the type of fraction that has antioxidant activity from *M. oleifera* leaf powder. The principle of the analytical method for determining the total flavonoid content is the reaction of the stable complex formation between aluminum chloride and the keto group on the C-4 atom and the hydroxy group on the C-3 or C-5 atom, which is adjacent to the flavone or flavonol group [14]. Total flavonoid content is determined using a spectrophotometer at a wavelength of 415 nm. The absorbance value of the quercetin standard solution was made in a calibration curve with a linear regression equation: $y = 0.009x + 0.0124$, with a correlation coefficient value ($R^2$) of 0.9989. The determination of the total phenolic content of each of the *M. oleifera* leaf extracts is displayed in Table 3.

Based on the determination results of the total flavonoid content of each fraction, it is known that the ethyl acetate fraction extract has the highest flavonoid content, which is $58.6389 \pm 2.051\%$. Compared to the methanol and n-hexane fraction extracts, the methanol fraction extract produced $48.9056 \pm 0.0809\%$, while the n-hexane fraction extract yielded $3.3056 \pm 0.039\%$. Thus, the ethyl acetate fraction extract produced the most flavonoid compounds.

Contrary to Kefayati et al. [17], who reported that total methanolic extract showed the highest total flavonoid compounds values, which was $208.23 \pm 0.007$ mg/gm, while the ethyl acetate fraction yielded $65.80 \pm 0.006$ mg/gm, lower than that of the methanol fraction. Rahman et al. [18] reported that the extraction of flower moon leaves (*T. diversifolia*) using ethanol as a solvent produced $3.41$ mg/gm, while ethyl acetate as a solvent produced flavonoid compounds of $2.21$ mg/gm.

This study aims to determine the ability of *M. oleifera* leaf extract, which contains a group of phenolic and flavonoid compounds, to have an antioxidant activity that inhibits free radicals. It is affected by the hydrogen atoms in the hydroxy groups in the core of phenolic and flavonoid compounds, which act as hydrogen atom donors or free-radical fighters through an electron transfer process [20]. This causes the phenolic compounds to change into phenoxyl compounds (Fig. 1) through a rearrangement.

The preliminary tests conducted to determine the total phenolic and total flavonoid content contained in the *M. oleifera* leaf sample provide information that the crude extract from the ethyl acetate fraction has the highest total phenolic and total flavonoid values among the methanol and n-hexane fractions of $46.5489 \pm 1.832$ and $58.6389 \pm 2.051$, respectively. This demonstrates that the compound

| Fractions | Absorbance | Phenolic levels (%w/w) | Average + SD |
|-----------|------------|------------------------|--------------|
| n-hexane  | 0.0403     | 1.7148                 | 1.4595 ± 0.361 |
| Ethyl acetate | 2.1269   | 45.2532                | 46.5489 ± 1.832 |
| Methanol  | 1.8419     | 39.1894                | 39.74574 ± 0.786 |

Laboratory analysis of UPI, 2021.
types of phenolic and flavonoid isolated in the ethyl acetate fraction are a group of semi-polar compounds. The study by Kefayati et al. [17] reported that the phenolic compounds have the strongest antioxidant activity in inhibiting free radicals. Moreover, Kumbhare et al. [16] said that antioxidant activity correlates with the content of total phenolic compounds. Methanolic, chloroform, and petroleum ether extracts at various concentrations ranging from 25 to 100 µg/ml were tested for their antioxidant activity using the DPPH radical scavenging assay method.

Antioxidant activity test of ethyl acetate fraction using DPPH method

Based on the information obtained, the determination of total phenolic and total flavonoid content has been conducted. The antioxidant activity testing focuses on the ethyl acetate fraction. The ethyl acetate fraction has a relatively high total phenolic and flavonoid content compared to the other fractions. The antioxidant activity testing was carried out using the DPPH method. The antioxidant activity testing on the crude extract, a fractionated result, is one of the primary test methods that provides information regarding the ability of natural compound extracts to inhibit free radicals (DPPH) by using a comparison compound with a quercetin-standardized solution. The determination of antioxidant activity is a quantitative test by capturing free radicals contained in DPPH compounds by the compounds that have a substituted-OH (hydroxy) group in the benzene core contained in natural material compounds, such as those in the group of phenolic and flavonoid compounds, using a UV-Vis spectrophotometer. The absorbance value resulting from the measurement of the UV-Vis spectrophotometer is converted into an IC₅₀ value (inhibitory concentration), defined as the test compound

| Fractions   | Absorbance | Flavonoid levels (% w/w) | Average + SD       |
|-------------|------------|--------------------------|--------------------|
| n-hexane    | 0.0419     | 3.2778                   | 3.3056 ± 0.039     |
|             | 0.0424     | 3.3333                   |                    |
| Ethyl acetate | 0.5532   | 60.0889                  | 58.6389 ± 2.051    |
|             | 0.5271     | 57.1889                  |                    |
| Methanol    | 0.4577     | 49.4778                  | 48.9056 ± 0.0809   |
|             | 0.4474     | 48.3333                  |                    |

| Laboratory analysis of LIPI, 2021.

Figure 1. Formation, incorporation, and rearrangement reactions of phenoxy radicals [40].
concentration that can capture or stabilize free radicals as much as 50%. The smaller the IC<sub>50</sub> value is, the more active the test compound will be against the antioxidant activity test. The graph showing the percentage of the inhibition versus the concentration of ethyl acetate, methanol, and n-hexane fraction extracts can be seen in Figures 2, 3, and 4 and Table 4. Table 4.

Based on the graph, it is known that the regression equation value is \( y = 0.2757x + 23.337 \), with a correlation coefficient \( R^2 \) value of 0.8797. The IC<sub>50</sub> value obtained using the regression equation formula is 30.309 mg/ml. The IC<sub>50</sub> value obtained from this experiment is relatively low, below 50 mg/ml, proving that the ethyl acetate fraction extract from <i>Moringa oleifera</i> leaf powder is strong enough to be used as an antioxidant activity [21]. The methanol fraction extract IC<sub>50</sub> value was 80.8865 and the n-hexane fraction extract IC<sub>50</sub> value was 155.3556. Atawodi et al. [22] reported that using the xanthine oxidase model system, extracts of roots, leaves, and stem bark of <i>M. oleifera</i> were extracted using methanol solvent and exhibited strong in vitro antioxidant activity with IC<sub>50</sub> values of 16, 30, and 38 µl, respectively. Differences in the antioxidant content of <i>M. oleifera</i> were different due to differences in solvents due to varying types of antioxidants such as ascorbic acid, β-carotene, kaempferol, quercetin, rutin, isothiocyanates derived from leaves, tocopherol, myricetin, and lectins derived from seeds, palmitic acid, and phytosterols of flowers [23]. Kumbhare et al. [16] found that the bark extract of <i>M. oleifera</i> with a DPPH reagent of 78.49% produced the most antioxidant activity with a methanol solvent, followed by a chloroform fraction extract of 50.68% and a petroleum ether extract of 34.14%.

In poultry, antioxidant compounds help cope with stress [24–28], especially in countries with tropical climates, such as Indonesia and Southeast Asian countries, that consist of two seasons, namely dry and rainy seasons. In the dry season, high environmental temperatures cause poultry to experience stress (heat stress) [29,30]. To reduce this situation, poultry requires the intake of foods that contain antioxidants, such as vitamin C, and
active compounds derived from plants or other natural ingredients [28,31,32]. Antioxidants in M. oleifera leaves are useful in reducing the stress conditions in this poultry, especially in broilers prone to stress due to extreme changes in environmental temperature. In goats, it was reported that M. oleifera leaves help increase ration consumption and the immune system through the transfer of bioactive compounds, especially antioxidants and vitamin C, in the milk [33]. In humans, M. oleifera leaf extract is pharmacologically helpful in medicine because it has anti-inflammatory, antioxidant, antitumor, anticancer, as well as antibacterial and antifungal activities [34]. The DPPH method is used to test the antioxidant activity of the ethyl acetate fraction. The IC₅₀ value is found in Table 4.
β-carotene content

Isolation of carotenoid compounds from *M. oleifera* leaf powder is important to determine the number of carotenoid compounds contained in *M. oleifera* leaf flour, which is as much as 0.4798 gm. Acknowledging the β-carotene content in the samples of *M. oleifera* leaf powder can function as additional nutrition and antibody enhancement for poultry as β-carotene is a source of pro-vitamin A [33–36]. β-carotene can also improve the reproductive performance of livestock, and the high content of antioxidant compounds will make livestock more resistant to disease [37–39]. In the form of an orange-red powder, the isolated carotenoid compound provides an observation. This study found that the β-carotene content in a 50-gm sample of *M. oleifera* leaf powder was 0.956%. Based on the calculation, the result is as follows:

\[
\% \text{ Carotenoids} = \frac{0.4798 \text{ gm}}{50 \text{ gm}} \times 100 = 0.956
\]

**Conclusion**

From the research, *M. oleifera* leaves are proven to have fairly high antioxidant activity, which is 30.309 mg/ml. This is because the *M. oleifera* leaves contain potential compounds that function as inhibitors of antioxidant activity, which are the groups of phenolic and flavonoid compounds. Phenolic and flavonoid compounds contained in the samples of *M. oleifera* leaf powder are determined by calculating the total phenolic content and total flavonoid content, which are 46.5489 ± 1.832 (%w/w) and 58.6389 ± 2.051 (%w/w), respectively.

**List of abbreviations**

DPPH, 2,2-Diphenyl-1-picrylhydrazyl; IC, Inhibitory concentration; UV, Ultraviolet; nm, Nanometer; TLC, Thin-layer chromatography; rpm, Revolutions per minute; mM, Millimolar; AOAC, Official method of analysis; μL, Microliter; R², Correlation coefficient value; %w/w, Percentage of weight per weight.

**Acknowledgment**

The authors would like to thank the Faculty of Animal Science, Jambi University, for funding research for the Hibah applied research project 2020.

**Conflict of interest**

The authors declare that they have no conflict of interest.

**Authors’ contributions**

UH and S designed the study. KK was involved in laboratory work, interpreted the data, and drafted the manuscript. AB and Al took part in the critical checking of this manuscript. All the authors read and approved the publication of this article.

**References**

[1] Susilowati A, Elfatti D, Rachmat HH, Yulita KS, Hadi AN, Kusuma YS, et al. Vegetation structure and floristic composition of tree species in the habitat of scaphium macropodum in Gunung Leuser National Park, Sumatra, Indonesia. Biodiv J Biol Div 2020; 21(7):3025–3033. https://doi.org/10.13057/biodiv/d210720

[2] Ogale SC, Kasture SB, Kasture VS, Tiwari R, and Zaid T. Screening of methanolic extract of *Sterculia scaphigera* wall seeds for ulcerprotective and antioxidant activity. J Pharm Sci 2015; 4(1):1332–46.

[3] Susilowati A, Hendalastuti RH, Elfatti D, and Hasibuan MH. The composition and diversity of plant species in Pasak Bumi’s (Eurycoma longifolia) habitat in batang labu sutem forest, North Sumatra, Indonesia. Biodiv J Biol Div 2019; 20(2):413–8. https://doi.org/10.13057/biodiv/d200215

[4] Yameogo CW, Bengaly MD, Savadogo A, Nikiema PA, Traore SA. Determination of chemical composition and nutritional values of *Moringa oleifera* leaves. Pak J Nutr 2011; 10(3):264–8.

[5] Kholfi A, Morsy A, Gouda G, Anele U, ML GML. Effect of feeding diets with processed *Moringa oleifera* meal as protein source in lactating anglo-nubian goats. Anim Feed Sci Technol 2016; 217(6):45–55. https://doi.org/10.1016/j.anifeedsci.2016.04.012

[6] Kholf AF, Gouda GA, Morsy TA, Salem AZM, Lopez S, Kholf AlM. *Moringa oleifera* leaf meal as a protein source in lactating goat’s diets: feed intake, digestibility, ruminal fermentation, milk yield and composition, and its fatty acids profile. Small Rum Res 2015; 129(8):129–37.

[7] Misra S, Misra MK. Nutritional evaluation of some leafy vegetable used by the tribal and rural people of South Odisha, India.

[8] Babiker EE, Juhaimein FAI, Ghafoor K, Abdoun KA. Comparative study on feeding value of moringa leaves as a partial replacement for alfalfa hay in ewes and goats. Livest Sci 2017; 195:21–26; https://doi.org/10.1016/j.livsci.2016.11.010

[9] Hassan MA, Xu T, Tian Y, Zhong Y, Ali FAZ, Yang X, et al. Health benefits and phenolic compounds of *Moringa oleifera* leaves: A comprehensive review. Phytomedicine 2021; 93(12):153771; https://doi.org/10.1016/j.phymed.2021.153771

[10] Foild N, Makkar H, Becker K. The potential of *Moringa oleifera* for agricultural and industrial uses.

[11] Reddy BA, Reddy NP, Gunasekar D, Blond A, Bodo B. Biflavonoids from Occhna Lanceolata. Phytochem Lett 2008; 1(1):27–30; https://doi.org/10.1016/j.phytochem.2007.12.005

[12] Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (Kakinoha-Cha). Food Chem 2005; 89(4):569–75; https://doi.org/10.1016/j.foodchem.2004.03.013

[13] Yen G-C, Chen H-Y. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J Agric Food Chem 1995; 43(1):27–32; https://doi.org/10.1021/jf00049a007

[14] Rodriguez-Amaya DB. A guide to carotenoid analysis in foods. International Life Sciences Institute (ILSI) Press, Washington, DC. AOAC. Official Method of Analysis. 18th edition, Association of Official Analytical Chemists, Washington, DC, 2005.

[15] Kumhare A, Guleha V, Sivakumar T. Estimation of total phenolic and flavonoid contents, cytotoxicity and in vitro antioxidant activity of stem bark of *Moringa oleifera*. Asian Pac J Trop Dis 2012; 2(2):144–50; https://doi.org/10.1016/j.aptd.2012.01.003-4

[16] Kefayati Z, Motamed S, Shojaai A, Noori M, Ghods R. Antioxidant activity and phenolic and flavonoid contents of the extract and subfractions of *Euphorbia splendida* Mobayen. Pharmacog Res 2017; 9(4):362; https://doi.org/10.4103/prpr.12_17

[17] Rahman NF, Nursamsiar N, Megawati M, Handayani H, Suares CA. Total phenolic and flavonoid contents and antioxidant activity of
Tithonia diversifolia and (Hemsley). Indones J Pharm Sci Technol 2022; 1(1):57; https://doi.org/10.24198/ijpstk.v1i1.36900

[19] Esmaeli AK, Taha BM, Mohajer S, Banisalam B. Antioxidant activity and total phenolic and flavonoid content of various solvent extracts from in vivo and in vitro grown trifolium pratense L (Red Clover). BioMed Res Int 2015; 2015:1–12; https://doi.org/10.1155/2015/643285

[20] Janeiro P, Oliveira Brett AM. Catechin electrochemical oxidation mechanisms. Analyt Chim Acta 2004; 518(1–2):109–15; https://doi.org/10.1016/j.aca.2004.05.038

[21] Phongpaichit S, Nilkom J, Rungjindamai N, Sakayaroj J, Hutadilok-Towatana N, Rukchaisirikul V, et al. Biological activities of extracts from endophytic fungi isolated from garcinia plants. FEMS Immunol Med Microbiol 2007; 51(3):517–25; https://doi.org/10.1111/j.1574-695X.2007.00331.x

[22] Atawodi SE, Atawodi JC, Idakwo GA, Pfundstein B, Haubner R, Wurtele G, et al. Evaluation of the polyphenol content and antioxidant properties of methanol extracts of the leaves, stem, and root barks of Moringa oleifera Lam. J Med Food 2010; 13(3):710–16; https://doi.org/10.1089/jmf.2009.0057

[23] Singh AK, Rana HK, Tshabalala T, Kumar R, Gupta A, Ndhlala AR, et al. Phytochemical, nutraceutical and pharmacological attributes of a functional crop Moringa oleifera Lam: An overview. South Afr J Bot 2020; 129(3):209–20; https://doi.org/10.1016/j.sajb.2019.06.017.

[24] Wang L, Piao XL, Kim SW, Piao XS, Shen YB, Lee HS. Effects of Forsythia suspensa extract on growth performance, nutrient digestibility, and antioxidant activities in broiler chickens under high ambient temperature. Poult Sci 2008; 87(7):1287–94; https://doi.org/10.3382/ps.2008-00023

[25] Ramnath V, Rekha PS, and Sujatha KS. Amelioration of heat stress induced disturbances of antioxidant defense system in chicken by brahma rasayana. Evid-Based Compl Altern Med 2008; 5(1):77–84; https://doi.org/10.1093/ecom/mnl116

[26] Pamplona R, Costantini D. Molecular and structural antioxidant defenses against oxidative stress in animals. Am J Physiol-Regul Integr Comp Physiol 2011; 301(4):R843–63; https://doi.org/10.1152/ajpregu.00034.2011

[27] Akbarian A, Michiels J, Golian A, Buyse J, Wang Y, De Smet S. Gene expression of heat shock protein 70 and antioxidant enzymes, oxidative status, and meat oxidative stability of cyclically heat-challenged finishing broilers fed origanum compactum and curcuma xanthorrhiza essential oils. Poult Sci 2014; 93(8):1930–41; https://doi.org/10.3382/ps.2014-03896

[28] Akbarian A, Golian A, Kermanshahi H, De Smet S, Michiels J. Antioxidant enzyme activities, plasma hormone levels and serum metabolites of finishing broiler chickens reared under high ambient temperature and fed lemon and orange peel extracts and Curcuma xanthorrhiza essential oil. J Anim Physiol Anim Nutr 2015; 99(1):150–62; https://doi.org/10.1111/jpa.12188

[29] Ma D, Shan A, Chen Z, Du J, Song K, Li J, Xu Q. Effect of Ligustrum lucidum and Schisandra chinensis on the egg production, antioxidant status and immunity of laying hens during heat stress. Arch Anim Nutr 2005; 59(6):439–47; https://doi.org/10.1080/17450390500353499

[30] Lara L, Rostagno M. Impact of heat stress on poultry production. Animals 2013; 3(2):356–69; https://doi.org/10.3390/ani3020356.

[31] Maini S, Rastogi SK, Korde JP, Madan AK, Shukla SK. Evaluation of oxidative stress and its amelioration through certain antioxidants in broilers during summer. J Poult Sci 2007; 44(3):339–47; https://doi.org/10.2141/jpsa.44.339

[32] Akbarian A, Michiels J, Degroote J, Majededdin M, Golian A, De Smet S. Association between heat stress and oxidative stress in poultry; mitochondrial dysfunction and dietary interventions with phytochemicals. J Anim Sci Biotechnol 2016; 7(1):37; https://doi.org/10.1186/s40104-016-0097-5

[33] Al-Juhaimi FY, Alsaemahi ON, Abidoun KA, Ghafor K, Babiker EE. Antioxidant potential of moringa leaves for improvement of milk and serum quality of aardig goats” South Afr J Bot 2020; 129:134–7; https://doi.org/10.1016/j.sajb.2019.03.022

[34] Padayaciee B, Bainjath H. An updated comprehensive review of the medicinal, phytochemical and pharmacological properties of Moringa oleifera. South Afr J Bot 2020; 129(3):304–16; https://doi.org/10.1016/j.sajb.2019.08.021

[35] Olson JB, Ward NE, Koutsos EA. Lycopene incorporation into egg yolk and effects on laying hen immune function. Poult Sci 2008; 87(12):2573–80; https://doi.org/10.3382/ps.2008-00072

[36] Faulks RM, Southon S. Challenges to Understanding and Measuring Carotenoid Bioavailability. Biochim Biophys Acta- Mol Basis Dis 2005; 1740(2):95–100; https://doi.org/10.1016/j.biomolbasis.2004.11.012

[37] Suriy PF, Speake BK. Sparks NH. Carotenoids in avian nutrition and embryonic development 2 antioxidant properties and discrimination in embryonic tissues. J Poult Sci 2001; 38(2):117–45; https://doi.org/10.2141/jpsa.38.117

[38] Suriy PF. Natural antioxidants in avian nutrition and reproduction. Nottingham University Press, Nottingham, UK, 2002.

[39] Stahl W, Sies H. Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta 2005; 1740(2):101–7; https://doi.org/10.1016/j.bba.2004.11.012

[40] Bruneton J. Pharmacognosie-Phytochimie, Plantes Médicinales. Tec & Dac Éditions médicinales internationales, Paris, France.