

Chapter

Antioxidant Fortification of Eggs through Nutrition of Laying Hens Administered Herbs/Medicinal Plants

Habeeb O. Yusuf and Ruth T.S. Ofongo

Abstract

The sole aim of raising pullet hens in the poultry industry is to produce eggs for human consumption in a large scale when they commence laying. Eggs are important dietary components to humans both adult and children and is classified as complete protein. However, certain quality of eggs produced by laying hens is further influenced by the diet consumed which in turn is determined by the quality of the feed ingredients making up the diet. Antibiotic residue in eggs and antimicrobial resistance are few concerns to consumers of poultry products. The current era of limiting antimicrobial utilization for livestock production has increased research into medicinal plants and herbs as suitable alternative. Antioxidant and anti-inflammatory activities reported in literature indicate the invaluable benefits of these plants both for humans and livestock. This book chapter attempts to present the 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant scavenging activity of eggs from laying hens fed medicinal plants – *Vernonia amygdalina* and *Ocimum gratissimum* as component of feed or administered orally as an aqueous extract. The DPPH antioxidant scavenging activity was present in eggs sampled but was better ($p < 0.05$) in eggs of laying hens administered aqueous *O. gratissimum* extract.

Keywords: antioxidants, antioxidant scavenging activity, medicinal plants, eggs, nutrition, laying hens

1. Introduction

Healthy food and healthy diet are a major concern to health-conscious individuals; since health lost is wealth lost. The concern of such individuals cannot be over emphasized. The genetic modification of plants and animals is an evolving development which is yet to be generally accepted by consumers of agricultural products. The current shift towards natural sources of antioxidants reported [1] can be attributed to the unfavorable effects of synthetic sources of antioxidants resulting from prolonged usage.

Reactive oxygen species (ROS) otherwise referred to as free radicals are by-products unavoidably produced in biological systems in the course of normal cellular

energy function [1, 2]. They are also produced through exogenous sources such as environmental pollutants, radiation, pesticides [1, 3, 4] etc. just to mention a few. Free radicals are important for some biological functions physiologically by acting as cell signaling molecules which function against cellular responses as reported in literature [5, 6]. Oxidative stress elicits adverse effects on lipids, proteins and nucleic acids thereby resulting into a number of degenerative conditions [7–10]. Free radical scavengers or antioxidants delay/inhibit damage made to cells by converting ROS to non-reactive radical species [1]. Common antioxidants include vitamin E (α -tocopherol), vitamin C (ascorbic acid), and β -carotene [11, 12].

Generally speaking; consumption of antioxidant rich food by health-conscious individuals has more to do with preventing damage in tissues and privation of cellular functions resulting from free radicals' intermediates generated by cells during normal metabolism [2] or avoiding degenerative diseases. Antioxidant activities of plants are regarded as safe. This activity is attributed mainly to phenolic compounds [5, 13] which act as hydrogen donors, reducing agents, oxygen quenchers besides their metal chelating potential [3]. These properties play a significant role in neutralizing free radicals [3].

1.1 Eggs as quality food

Eggs are considered high quality protein which is readily digestible, however, with recent developments in human health, animal nutrition, it is gradually developing into a functional food for health and better wellbeing. The amino acid profile of eggs is adequate to meet both essential and non-essential amino acid needs of both adults and young children making it a complete protein. Eggs are also considered cheap, extremely nutritious, palatable and readily accessible across the globe [14, 15].

Egg yolk, egg white/albumen and egg shell with membrane respectively; accounts for approximately 27.5%, 63% and 9.5% of the whole egg [16]. The various constituent of egg nutrients are proteins and lipids 12% respectively; while the edible portion is made up of 74% water [17]. Eggs contain less than 1% carbohydrate along with vitamins and minerals [17–20]. Both high density and low-density lipoproteins in addition to livetin's is located in egg yolk. Ovalbumin, ovotransferrin, ovomucoid, ovomucin just to mention a few are protein fractions present in egg white [21]. The quality of egg protein is such that it is used as a golden standard for measuring the quality of other food proteins [18].

Greater part of lipids in egg yolk are present as triglycerides—almost 65%, however, carotenoids constitute less than 1% while phospholipids and cholesterol are 30% and 4% respectively [22]. Lipids from egg yolk have been used to supply long-chain polyunsaturated fatty acids, docosahexaenoic acid (DHA) and phospholipids incorporated into infant formula [23, 24]. They are also regarded as an excellent source of micronutrients—vitamins and minerals. According to published work [18] eggs contain approximately 16% required daily intake (RDI) of phosphorus. The RDI of eggs was reported for selenium (29%); iron (9%) and zinc (9%) respectively. In addition, eggs also provide 10% of the RDI for fat soluble vitamins, vitamin B2, B12, biotin and pantothenic acid [18]. As it is in spite of the nutritive quality of eggs, it appears the need to limit its cholesterol concentration, especially high-density lipoproteins are focused on meeting healthy outcomes for consumers of eggs. This has led to manipulating poultry diets to influence the nutritional constituents in eggs [25]. Several studies have been carried out in this regard and reported in literature [14, 15, 26–35]. Besides the fatty acid constituents of eggs, some minerals like selenium and iodine have been enriched in eggs via enrichment of feed [35, 36] and feed formulation [37].

Intake of antioxidants through diet is known to be important in reducing oxidative damage in cells and improving human health. Although eggs are known for their exceptional, nutritional quality, they are not generally considered as antioxidant foods [21].

1.2 Antioxidant content of eggs

Many of the compounds present in eggs—vitamin E, and A, selenium, phospholipids and carotenoids—show evidence of antioxidant properties; eggs are not normally considered as antioxidant foods [21]. Even though certain polyunsaturated fatty acids (PUFA) exhibit antioxidants properties; eggs are mostly consumed for their protein constituents and minerals which are components of egg.

Furthermore, it is important to note that with recent developments in enriching eggs via feed thereby making eggs as functional foods then consumption of eggs might just be a source of antioxidants for healthy living.

A recent report [38] testing the antioxidant property of a product containing pectic oligosaccharides, with prebiotic, chlorogenic as well as antioxidant effect on the possibility of enhancing egg laying performance and egg quality of laying hens. The results showed that the tested product enhances laying hens egg quality and performance, particularly by means of its antioxidant properties that play a part to sustain oxido-redox balance, consequently reducing the negative effects triggered by oxidation like degradation of egg quality [38]. By this, may the various nutrient components of egg which make up the quality of eggs can be improved upon by feed manipulation. The antioxidant properties of eggs are exhibited by the different components' present either in the egg yolk or egg white.

Ovalbumin, ovatransferrin, ovomucin, lysozyme, cystatin, in egg white reportedly have antioxidant properties. Phosvitin, phospholipids, carotenoids and vitamin E which are components of egg yolk.

Ovalbumin in egg white has free thiol groups that regulate redox status and bind metal ions thereby exerting antioxidant properties. In conjugation with saccharides increased antioxidant activity takes place [39, 40]. Ovomucin in egg white inhibit hydrogen peroxide H_2O_2 -induced oxidative stress in human embryonic kidney [41]. Furthermore, lysozyme in egg white suppress reactive oxygen species (ROS) and oxidative stress genes [42]. In the case of nutrient components with antioxidant properties in egg yolk; phospholipids are 10% of egg yolk dry matter. Hydrolyl amines in the side chains of egg yolk phospholipids play a role in radical scavenging with antioxidant properties [43]. The unsaturated backbone and aromatic rings of carotenoids present in egg yolk help in neutralizing singlet oxygen, free radicals what is more is protective against oxidative damage [44–46].

2. Antioxidant properties of medicinal plants

Current research thrust has exposed the numerous benefits of herbs and medicinal plants in the nutrition of farm animals. Needless to say, several herbs and medicinal plants formerly taken as traditional medicine have been used for animal feeding trials. In an earlier report [47, 48] a wide range of extracts from herbal plants have antioxidant properties.

In particular is extracts of herbs from the Labiatae family—oregano, thyme, basil, mint, rosemary, sage, savory, marjoram, etc. The antioxidant properties of hyssop and lavender were attributed to phenolic terpenoid compounds such as carvacrol, thymol, menthol and eugenol just to mention a few [2]. Evidence in literature regarding antioxidant properties of herbal plants [49] further revealed that both phenolic

and nonphenolic compounds exhibit antioxidant properties in them. Example of nonphenolic compounds with antioxidant properties include glycosides [50]. A recent report [49] showed the antioxidant properties of phenols from *Vernonia amygdalina* Delile. (Asteraceae). High levels of phenolic content 54.61 ± 0.94 mg GAE/g in *V. amygdalina* [49] has also been reported from phytochemical analysis of *Vernonia amygdalina*. The methanolic extract indicated that phenolic compounds were highly detected (+++) while flavonoids were detected (++) [51]. The medicinal plant *V. amygdalina* is rich in flavonoids, tannins and saponins, which may possibly play a part in anti-oxidative effect [49, 51]. From literature *V. amygdalina* has a flavonoid content of 22.53 ± 0.91 mg QE/g [49]. Phenolics hydroxy groups present in the molecular structure of flavonoids earlier stated as exhibiting antioxidant properties are involved in antioxidant properties of flavonoids. The powerful antioxidant property exhibited by phenolic compounds [52] present in plants constituents can be attributed to the hydroxyl groups [53] present in them. The medicinal plant *Ocimum gratissimum* also has both antioxidant anti-inflammatory properties already attributed to its therapeutic benefits from literature [54, 55]. The presence of other phytochemicals - saponins, terpenoids, glycosides and alkaloids—in aqueous *O. gratissimum* besides flavonoids and phenols (Ofongo, 2023, unpublished data). These phytochemicals may further play a part in its anti-inflammatory and anti-oxidative activities [54, 55].

2.1 Medicinal plants as component of feed and their benefits to nutrition of laying hens

Medicinal as component of feeds is becoming a common practice in the livestock industry either as suitable alternative to antibiotics or for their growth performance and health promoting benefits to livestock and ultimately to consumers of livestock consumers. Other benefits of medicinal plants as components of feed include; antimicrobial; anti-inflammatory; immune modulatory effect and antioxidant effect.

As earlier stated, the efficacy of herbal extracts as antioxidant feed additives needs to be evaluated and correlated with their phenolic content [2]. The use of medicinal to feed laying hens is more or less an evolving practice in the poultry industry.

Firstly, from improving productive performance, modulation of cholesterol contents in eggs and egg quality have been reported [14, 15, 34, 38, 56, 57]. However, gradually, inclusion of medicinal plants in diets of laying hens is focusing at making eggs functional food by improving or fortifying the content of nutrients present in eggs with benefits to consumers of eggs such as antioxidant properties [34, 58–60].

This book chapter tries to illustrate possibility for antioxidant scavenging activity enhancement in eggs from laying hens by administering medicinal plants via feed or orally.

3. Materials and methods

3.1 Collection and identification of plant materials

Two medicinal plants (*Vernonia amygdalina* Ochile (Compositae) NDUP/21/14—**Figure 1** and *O. gratissimum* L. Lamiaceae) NDUP/12/13—**Figure 2**) were used in this study. Authentication of the plants were done at Herbarium Unit of the Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University. Fresh leaves of *V. amygdalina* and *O. gratissimum* were collected from Niger Delta University Teaching and Research Farm, Wilberforce Island, Bayelsa State, Nigeria.



Figure 1.
V. amygdalina.



Figure 2.
Ocimum gratissimum.

3.2 Preparation of extracts and chaff

A large amount of each leaf was collected either first thing in the morning or late in the evening. The leaves were separated from the stalk and placed in a large container filled with clean drinkable water into which little salt to brine the water. This was done to remove dust and debris from the leaves. Thereafter, the leaves were placed in a sieve to remove the brine and rinsed in clean drinkable water without salt again to remove any brine water on the leaves. The leaves were afterwards placed in

a sieve to drain out excess water. Each leave was chopped separately into fine particle size then one thousand gram (1000 g) of each leave was weighed separately for milling to obtain the aqueous extract. Seven hundred and fifty mills (750 ml) of clean drinkable water were used to mill 1000 g of each leave sample separately by means of an electric milling machine. The aqueous filtrate was obtained by passing the milled product through a cheese cloth. The obtained chaff was set aside, air dried then packaged in zip lock bag (**Figure 3A**) for use as component of feed (**Figure 3B**). The aqueous extract was administered to 22 weeks old bovan brown layer at an inclusion rate of 1 ml/bird administered twice a week. The obtained dried chaff was further milled to obtain fine particles and added to standard layers mash at an inclusion rate of 50 g/kg of complete feed. Feed mixed with chaff not for immediate use were stored (**Figure 4**).

3.3 Animal experiment

A feeding trial was carried out to access and determine improved DPPH concentration in eggs from laying hens administered medicinal plants either as component of feed of as aqueous extract. The experiment was arranged as a complete randomized



Figure 3.
Dried chaff of leaves in zip lock bag (A); fine milled chaff mixing into feed (B).



Figure 4.
Feed incorporated with chaff packaged in zip lock bag.

design having five (5) treatment groups of five (5) replicates and four (4) birds per replicate. A total of 100 bovan brown laying hens were purchased at 20 weeks of age randomly distributed to the above stated design then allowed to acclimatize for 2 weeks under the experimental treatments. Experimental collection of eggs for sampling commenced at week 22. The experiment was terminated after eight (8) weeks on day 56. Birds allocated to treatment 1 (T1) served as the control group. They were fed a standard layer's mash but where not administered aqueous plant extract neither was their feed supplemented with the chaff of *V. amygdalina* or *O. gratissimum*. Birds allocated to treatment 2 (T2) were fed diet supplemented with *V. amygdalina* chaff while birds in treatment 3 (T3) were administered 1 ml/bird of aqueous *V. amygdalina* twice a week. Birds assigned to treatment four (T4) had their diet supplemented with *O. gratissimum* chaff while birds in treatment five (T5) were administered 1 ml/bird of aqueous *O. gratissimum* extract twice a week.

3.4 Sample collection

On day 56, one egg per replicate was collected to determine antioxidant scavenging activity in inhibiting DPPH according to procedure illustrated below. The collected eggs were first cracked, homogenized then lyophilized to obtain a powder version of each egg before carrying out antioxidant scavenging activity in inhibiting DPPH using ascorbic acid as standard.

3.5 Proximate composition of feed and antioxidant scavenging activity in inhibiting DPPH

Samples of feed already supplemented with the respective chaff of *V. amygdalina* and *O. gratissimum* was collected into sample containers (100 g) for proximate analysis of feed as well as antioxidant activity in scavenging DPPH. The aqueous extract (30 ml) of either plant was also collected into sample bottles for antioxidant activity analysis in scavenging DPPH. Proximate composition of the experimental diets with and without each leave chaff as well as each respective chaff alone was carried out according to AOAC method.

Antioxidant activity of the plant extracts and feed supplemented with plant chaff in scavenging DPPH were evaluated on the basis of free radical scavenging effect of stable 2,2-diphenyl-1-picrylhydrazyl (DPPH). This was evaluated in comparison with Ascorbic acid standard, using a slightly modified method [61]. The compound DPPH is a synthetic compound not occurring in nature but it is utilized to evaluate the antioxidant activity of organic compounds such as vitamins, polyphenols and other phytochemicals.

Standard concentrations of Ascorbic acid standard were prepared at concentrations of 20, 40, 60, 80, and 100 µg/ml; respectively from a stock solution in triplicates using Methanol. Thereafter, a solution of DPPH was prepared using 0.1 mM of DPPH in methanol of which 2 ml of this solution was mixed with 3 ml of the test and standard solutions in test tubes. The solutions were shaken, then allowed to stand for 30 min in the dark before absorbance was measured at 517 nm using UV-VIS Spectrophotometer (Biomate 3, USA).

A standard control was prepared by mixing Methanol (3 ml) with 2 ml DPPH solution (0.1 mM, 1 ml). Methanol was used as a blank. The same procedure carried out with the Ascorbic acid standard was repeated with the test samples (leave chaff, aqueous extract and egg samples).

Percentage inhibition of DPPH was carried out using the formula below:

$$\text{Inhibition of DPPH} = (Ac - Aa) / Ac \times 100\%$$

Ac: absorbance of control sample.

Aa: absorbance of test samples or standard.

3.6 Statistical analysis

Collected data on DPPH scavenging activity in each sample (leaf chaff, aqueous extract, feed supplemented with leaf chaff and eggs) were subjected to analysis of variance (ANOVA). Statistically significant means were separated with Duncans Multiple Range test [62].

4. Results and discussions

4.1 Proximate composition of experimental diets

The proximate composition of layers mash fed to experimental birds as well as the proximate composition of *V. amygdalina* and *O. gratissimum* chaff is presented below in **Table 1**.

Crude protein concentration in *O. gratissimum* and *V. amygdalina* chaff was within the same range 12.00–13.00 g/kg DM. Ash concentration of *V. amygdalina* chaff was numerically higher (12.48 g/kg DM) than in *O. gratissimum* (9.17 g/kg DM). Dry matter concentration (943.15 g) was higher in *V. amygdalina* than in *O. gratissimum* (929.05 g). Proximate composition of feed showed an adequate crude protein concentration in the feed. Nitrogen free extract (carbohydrate) concentration was 61.61 g/kg DM in *O. gratissimum* which was numerically higher than value recorded in *V. amygdalina* and layers mash used in this study.

The synthetic compound 2,2-diphenyl-1-picrylhydrazyl (DPPH) is used as a reagent in antioxidant assays. It is used to evaluate the antioxidant activity of organic compounds such as vitamins, polyphenols and other phytochemicals. The addition of an organic compound to a solution of DPPH is used to measure the antioxidant activity of the compound. This activity is dependent on the ability of the compound to scavenge DPPH radical thereby limiting the ability of DPPH to absorb light at 517 nm. The lesser the absorbance of the solution, the higher the antioxidant scavenging

Nutrient	Layers mash	<i>Ocimum gratissimum</i> chaff	<i>V. amygdalina</i> chaff
Dry matter (gm)	926.80	929.05	943.15
Moisture (gm)	73.20	70.45	54.45
Crude protein	15.79	12.29	12.13
Crude fibre	14.88	12.01	12.33
Ash	10.76	9.17	12.48
Ether extract	5.79	4.90	5.86
NFE	52.78	61.63	57.20

NFE: nitrogen free extract.

Table 1.

Proximate composition of layers mash and leaf chaff (g/kg DM except otherwise stated).

activity of the test material. The DPPH scavenging activity is reported as percentage DPPH inhibition by the test compound. The lower the absorbance, the higher the percentage inhibition or free radical scavenging activity.

The antioxidant scavenging activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) by medicinal plants administered to layers or supplemented into complete feed is presented in **Table 2** below. Based on the DPPH scavenging activity (UV-absorbance at 517 nm); absorbance of DPPH was significantly ($p < 0.05$) low at 10 ug/ml concentration in *O. gratissimum* aqueous extract. The higher concentration resulted in significantly lower ($p < 0.05$) DPPH scavenging activity in *V. amygdalina* and *O. gratissimum* either in chaff or aqueous extract.

Table 3 shows the DPPH scavenging activity of *V. amygdalina* was high compared to *O. gratissimum* either as an aqueous extract or chaff. Values recorded was comparable to value obtained in Ascorbic acid standard. As indicated in **Table 3**: the least absorbance closest to value obtained using ascorbic acid was at a concentration of 200 ug/ml. The herb, *V. amygdalina* has antioxidant properties [63, 64].

The DPPH scavenging activity (% inhibition) is presented in **Figure 5**. *Vernonia amygdalina* chaff earlier reported in **Table 3** above elicited DPPH scavenging activity which was close to Ascorbic acid was significantly higher than values recorded for *O. gratissimum* leave chaff or aqueous extract. This value was also higher than that recorded for *V. amygdalina* aqueous extract in a dose dependent manner.

Expectedly, one will think the same trend will follow in DPPH scavenging activity for eggs collected from laying hens fed medicinal leaf chaff as component of feed or administered aqueous extract of either medicinal plant (**Table 4**). Rather; it was eggs from laying hens administered aqueous *O. gratissimum* that had numerically higher

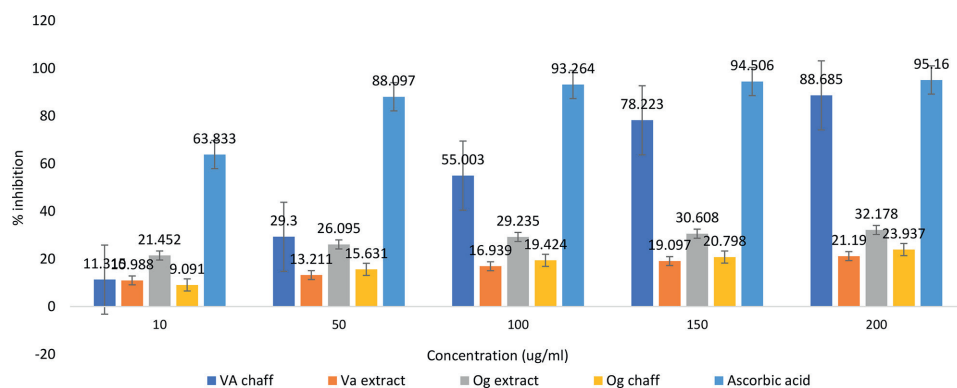
Concentration (ug/ml)	<i>V. amygdalina</i> (AE)	<i>V. amygdalina</i> (chaff)	<i>Ocimum gratissimum</i> (AE)	<i>O. gratissimum</i> (chaff)	SEM
10	0.454 ^b	0.452 ^c	0.400 ^d	0.463 ^a	0.001
50	0.442 ^a	0.360 ^d	0.377 ^c	0.430 ^b	0.001
100	0.423 ^a	0.229 ^d	0.361 ^c	0.411 ^b	0.001
150	0.412 ^a	0.112 ^d	0.354 ^c	0.404 ^b	0.001
200	0.402 ^a	0.058 ^d	0.346 ^c	0.388 ^b	0.001

abcd: means along the same column with different superscripts are significantly different ($p < 0.05$); DPPH: 2,2-diphenyl-1-picrylhydrazyl; AE: aqueous extract; SEM: standard error of mean.

Table 2.
DPPH scavenging activity (UV – Absorbance at 517 nm) in medicinal plants – Aqueous extract and chaff.

Concentration (ug/ml)	<i>V. amygdalina</i> (chaff)	Ascorbic acid
10	0.452	0.184
50	0.360	0.061
100	0.229	0.034
150	0.112	0.028
200	0.058	0.025

Table 3.
DPPH scavenging activity (UV-absorbance at 517 nm) in *V. amygdalina* chaff compared to ascorbic acid standard.

**Figure 5.**

DPPH scavenging activity (% inhibition). VA: *Vernonia amygdalina*; VA extract: *Vernonia amygdalina* aqueous extract; Og extract: *Ocimum gratissimum* Aqueous extract; Og chaff: *O. gratissimum* Chaff.

Treatment	Concentration (ug/ml)				
	10	50	100	150	200
Control group	8.044 ± 0.173	9.222 ± 0.065	10.922 ± 0.196	13.342 ± 0.285	16.285 ± 0.173
<i>V. amygdalina</i> chaff	8.829 ± 0.398	11.315 ± 0.113	12.230 ± 0.285	13.604 ± 0.173	13.996 ± 0.236
<i>Ocimum gratissimum</i> aqueous extract	9.287 ± 0.131	10.203 ± 0.131	12.165 ± 0.173	15.631 ± 0.196	17.332 ± 0.173
Ascorbic acid	63.833 ± 0.458	88.097 ± 0.429	93.264 ± 0.065	94.506 ± 0.227	95.160 ± 0.065

Table 4.

DPPH scavenging activity (% inhibition) in eggs from laying hens fed or administered medicinal plants.

DPPH scavenging activity which was better than that recorded for birds feed the layers mash control diet. Although values obtained in the study were lower than that recorded for Ascorbic acid; it can be said that may be the antioxidant components in *O. gratissimum* were better absorbed and incorporated into eggs of layers administered *O. gratissimum* aqueous extract than the chaff and *V. amygdalina* either as component of feed or aqueous extract for laying hens. The compound DPPH does not occur naturally and is not present in any organic compound; however, it is readily used to evaluate the antioxidant activity of organic compounds such as vitamins, polyphenols and other phytochemicals which can be present in medicinal plants and their extracts.

Natural products such as sesquiterpenoids and flavonoids were reported to be potential antioxidants [65–67]. These products are naturally obtained from food and use of medicinal plants. This fact further strengthens the antioxidative potential of *V. amygdalina*. Furthermore, results of earlier study added that not only flavonoids are responsible for any antioxidant effects of this plant species [68] but the presence of sesquiterpene lactones [69] might also play a part. Phytochemical screening of bitter leaf extract revealed a high concentration of flavonoids reported to be as the most abundant phytochemical present [63]. Antioxidant assay also indicated high levels of antioxidant activity and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity which was concentration dependent.

The possibility of enhancing egg quality of laying hens by means of utilizing products exhibiting antioxidant properties can contribute to maintain oxido-redox balance thereby reducing the effect of oxidation on egg quality—degradation of eggs [38].

Possibly consumption of medicinal plants by laying hens either as feed component or aqueous extract can fortify the antioxidant properties of eggs. Poultry feed is composed mostly of plant ingredients which can be attributed to the antioxidant scavenging activity recorded in eggs from laying hens fed the control diet. Besides spoilage of eggs which is a concern to both consumers and producers of table eggs, the possibility of improving the not only the shelf life [38] but also improvement in good egg quality parameters [14] as well as designing eggs as functional foods.

Increased content of optimal ω -3 fatty acids, better ω -6/ ω -3 fatty acids ratio, as well as good sensory profile of eggs optimal yolk color [14]. Furthermore, it has been shown in previous studies that fatty acid composition of eggs is dependent on fatty acid composition of feed given to laying hens which is subsequently transferred to the eggs [31].

A significant dose dependent DPPH scavenging activity (UV-absorbance at 517 nm) was observed in either medicinal plant consumed by laying hens and in eggs collected from the laying hens irrespective of treatment as presented in **Table 5**.

Table 6 further corroborated the possibility of plant to influence antioxidant scavenging activity of eggs from laying hens. The UV-absorbance at 517 nm for DPPH scavenging activity from eggs sampled showed significant improvement in antioxidant scavenging activity treatment 5 as earlier indicated in **Table 4**. Eggs from birds administered aqueous *O. gratissimum* significantly improved % inhibition of DPPH.

The possibility of producing functional eggs from laying hens for human consumption is wide. Apart from improving egg quality [38, 58], shelf life [38]; cholesterol content [57, 58], fatty acids profile and sensory profile [14, 15]. opportunities exist to improve antioxidant properties of eggs using medicinal plants. With increasing demand for enriched and functional foods which will provides various benefits to human health, eggs can be enriched with desirable nutrients [15] by means of dietary manipulation to achieve this goal [26, 27].

The all-important role of certain fatty acids such as linoleic and α -linolenic besides their long-chain (LC) *n*-6 and *n*-3 polyunsaturated fatty acids (PUFA) for humans have been reported some of the benefits ascribed to consumption of *n*-3 PUFA enriched eggs [15, 29]. Evidently this is made possible by feeding laying hens with

Concentration	Plants	Eggs
10ug/ml	0.443 ^a	0.467 ^a
50ug/ml	0.402 ^b	0.460 ^b
100ug/ml	0.356 ^c	0.454 ^c
150ug/ml	0.320 ^d	0.443 ^d
200ug/ml	0.298 ^e	0.433 ^e
SEM	0.001	0.001

abcde: means along the same column with different superscripts are significantly different ($p < 0.05$); DPPH: 2,2-diphenyl-1-picrylhydrazyl; SEM: standard error of mean.

Table 5.
DPPH scavenging activity (UV – Absorbance at 517 nm in plants and eggs sampled.

Treatment	Eggs
T1	0.451 ^{bc}
T2	0.449 ^c
T3	0.456 ^a
T4	0.453 ^b
T5	0.443 ^d
SEM	0.001

abcd: means along the same column with different superscripts are significantly different ($p < 0.05$); DPPH: 2,2-diphenyl-1-picrylhydrazyl; SEM: standard error of mean; T1: control group; T2: treatment 2 – *V. amygdalina* chaff; T3: treatment 3 – 1 ml aqueous *V. amygdalina*; T4: Treatment 4 – *Ocimum gratissimum* chaff; T5: Treatment 5 – 1 ml *O. gratissimum* aqueous extract.

Table 6.

Effect of treatment on DPPH scavenging activity (UV – Absorbance at 517 nm) in eggs from layers administered medicinal plant extract.

different by-products rich in PUFA [15, 27, 28, 31] such as flaxseed, rapeseed, microalgae, canola, chia (seed, meals or oils) etc.

Eggs for human consumption can have their fatty acid components enhanced by manipulating their feed by means of medicinal plants having antioxidant properties which may be transferred into the eggs. Furthermore, such eggs can serve as functional food for human consumption due to health benefits ascribe to eggs from laying hens fed with certain plants cum medicinal plants. In addition, the two medicinal plants reported here have antioxidant properties which can be of health benefit to consumers.

5. Conclusion

Although there are several variables for measuring antioxidant properties of medicinal plants; however, this study only looked at DPPH scavenging activity in eggs from laying hens fed *V. amygdalina* and *O. gratissimum* leaf chaff as components of the feed. It also evaluated DPPH scavenging activity when the aqueous extract of both plants is administered to laying hens. The DPPH scavenging activity of *V. amygdalina* chaff was high and comparable to ascorbic acid; but it was aqueous *O. gratissimum* administration to laying hens that yielded improved DPPH scavenging activity.

Acknowledgements

Miss Dressman, Mary Paul is gratefully acknowledged for collecting the eggs and participating in the study as part of her BSc project.

Conflict of interest

The authors declare no conflict of interest.

Author details

Habeeb O. Yusuf¹ and Ruth T.S. Ofongo^{2*}

1 Faculty of Pharmacy, Department of Pharmacognosy, University of Lagos,
Lagos State, Nigeria

2 Faculty of Agriculture, Department of Animal Science, Niger Delta University,
Wilberforce Island, Bayelsa State, Nigeria

*Address all correspondence to: ruthofongo@ndu.edu.ng; ruthofongo@gmail.com

IntechOpen

© 2023 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Lourenço SC, Moldão-Martins M, Alves VD. Antioxidants of natural plant origins: From sources to food industry applications. *Molecules* (Basel, Switzerland). 2019;**24**(22):4132. DOI: 10.3390/molecules24224132
- [2] Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M, Derakhshan M. Thymol and carvacrol supplementation in poultry health and performance. *Veterinary Medicine and Science*. 2022;**8**:267-288. DOI: 10.1002/vms3.663
- [3] Fadzaï B, Elaine C, Stanley M. Evaluation of nitrite radical scavenging properties of selected Zimbabwean plant extracts and their phytoconstituents. *Journal of Food Processing*. 2014. Article ID 918018:7. DOI: 10.1155/2014/918018
- [4] Masoko P, Eloff JN. Screening of twenty-four south African *Combretum* and six *Terminalia* species (Combretaceae) for antioxidant activities. *African Journal of Traditional, Complementary and Alternative Medicines*. 2007;**4**:231-239. DOI: 10.4314/ajtcam.v4i2.31213
- [5] Huyut Z, Beydemir S, Gülçin I. Antioxidant and antiradical properties of selected flavonoids and phenolic compounds. *Biochemistry Research International*. 2017;**2017**, Article ID 7616791:10. DOI: 10.1155/2017/7616791
- [6] Comunian TA, Ravanfar R, de Castro IA, Dando R, Favaro-Trindade CS, Abbaspourrad A. Improving oxidative stability of echium oil emulsions fabricated by microfluidics: Effect of ionic gelation and phenolic compounds. *Food Chemistry*. 2017;**233**:125-134. DOI: 10.1016/j.foodchem.2017.04.085
- [7] Sagar BK, Singh RP. Genesis and development of DPPH method of antioxidant assay. *Journal of Food Science and Technology*. 2011;**48**(4):412-422. DOI: 10.1007/s13197-011-0251-1
- [8] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews*. 2010;**4**(8):118-126. DOI: 10.4103/0973-7847.70902
- [9] Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: Properties, sources, targets, and their implication in various diseases. *Indian Journal of Clinical Biochemistry*. 2015;**30**(1):11-26. DOI: 10.1007/s12291-014-0446-0
- [10] Haseeb A, Ghulam H and Imtiaz M. Antioxidants from Natural Sources. In *Antioxidants in Foods and Its Application*. Emad Shalaby and Ghada Mostafa Azzam Eds. Intech. 2018. DOI: 10.5772/intechopen.75961
- [11] Traber MG, Stevens JF. Vitamins C and E: Beneficial effects from a mechanistic perspective. *Free Radical Biology & Medicine*. 2011;**51**(5):1000-1013. DOI: 10.1016/j.freeradbiomed
- [12] Idamokoro EM, Falowo AB, Oyeagu CE, Afolayan AJ. Multifunctional activity of vitamin E in animal and animal products: A review. *Animal Science Journal*. 2020;**91**:e13352. DOI: 10.1111/asj.13352
- [13] Loi M, Paciolla C, Logrieco AF, Mulè G. Plant bioactive compounds in pre- and postharvest management for aflatoxins reduction. *Frontiers in Microbiology*. 2020;**11**:243. DOI: 10.3389/fmicb.2020.00243. PMID: 32226415; PMCID: PMC7080658
- [14] Spasevski N, Peulić T, Banjac V, Rakita S, Puvača N, Kokić B, et al.

Effects of adding the functional co-extrudates and natural pigments in the diet of laying hens on egg quality. In: 26th World's Poultry Congress, book of abstracts. 2020. p. 445

[15] Vlaicu PA, Panaite TD, Turcu RP. Enriching laying hens' eggs by feeding diets with different fatty acid composition and antioxidants. *Scientific Reports*. 2021;**11**:20707. DOI: 10.1038/s41598-021-00343-1

[16] Cotterill OJ, Geiger GS. Egg product yield trends from shell eggs. *Poultry Science*. 1977;**56**:1027-1031

[17] Li-Chan ECY, Kim HO. Structure and chemical composition of eggs. In: Mine Y, editor. *Egg Bioscience and Biotechnology*. Hoboken, NJ, USA: John Wiley & Sons, Ltd; 2008. pp. 1-95

[18] Seuss-baum I. Nutritional evaluation of egg compounds. In: Huopalahti R, López-Fandiño R, Anton M, Schade R, editors. *Bioactive Egg Compounds*. Berlin, Heidelberg, Germany: Springer; 2007. pp. 117-144. DOI: 10.1007/978-3-540-37885-3_18

[19] Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. *Journal of Agricultural and Food Chemistry*. 2005;**53**:8421-8431. DOI: 10.1021/jf050964f

[20] United States Department of Agriculture. United States Department of Agriculture: National Nutrient Database for standard reference Release 27. Available online: <http://ndb.nal.usda.gov/ndb/> [Accessed: February 25, 2023]

[21] Nimalaratne C, Bandara N, Wu J. Purification and characterization of antioxidant peptides from enzymatically hydrolysed chicken egg white. *Food Chemistry*. 2015;**188**:467-472. DOI: 10.1016/j.foodchem.2015.05.014

[22] Hatta H, Kapoor M, Juneja L. Bioactive components in egg yolk. In: Mine Y, editor. *Egg Bioscience and Biotechnology*. Hoboken, NJ, USA: John Wiley & Sons, Ltd.; 2008. pp. 185-237

[23] Carlson S, Montalto M, Ponder D. Lower incidence of necrotizing enterocolitis in infants fed a preterm formula with egg phospholipids. *Pediatric Research*. 1998;**44**:491-498. DOI: 10.1203/00006450-199810000-00005

[24] Hoffman DR, Theuer RC, Castaneda YS, Wheaton DH, Bosworth RG, O'Connor AR, et al. Maturation of visual acuity is accelerated in breast-fed term infants fed baby food containing DHA-enriched egg yolk. *Journal of Nutrition*. 2004;**134**:2307-2313. DOI: 10.1093/jn/134.9.2307

[25] Surai PF. Effect of selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. *British Poultry Science*. 2000;**41**:235-243. DOI: 10.1080/713654909

[26] Ao T et al. Effects of supplementing microalgae in laying hen diets on productive performance fatty-acid profile and oxidative stability of eggs. *Journal of Applied Poultry Research*. 2015;**24**(3):394-400. DOI: 10.3382/japr/pfv042

[27] Gonzalez-Esquerria R, Leeson S. Alternatives for enrichment of eggs and chicken meat with omega-3 fatty acids. *Canadian Journal of Animal Science*. 2001;**81**(3):295-305. DOI: 10.4141/A00-092

[28] Carrillo S et al. Potential use of seaweeds in the laying hen ration to improve the quality of n-3 fatty acid enriched eggs. In: *Nineteenth International Seaweed Symposium*. Dordrecht: Springer; 2008. pp. 271-278

- [29] FAO/WHO (Food and Agricultural Organization of the United Nations and World Health Organization). 2010 Fats and fatty acids in human nutrition. Report of an Expert Consultation Vol. 91 (FAO Food Nutrition Papers). 2010;**91**: 1-166. PMID: 21812367
- [30] Hayat Z, Cherian G, Pasha TN, Khattak FM, Jabbar MA. Oxidative stability and lipid components of eggs from flax-fed hens: Effect of dietary antioxidants and storage. *Poultry Science*. 2010;**89**(6):1285-1292. DOI: 10.3382/ps.2009-00256
- [31] Fraeye I, Bruneel C, Lemahieu C, Buyse J, Muylaert K, Foubert I. Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Research International*. 2012;**48**(2):961-969. DOI: 10.1016/j.foodres.2012.03.014
- [32] Shahidi F, Ambigaipalan P. Omega-3 polyunsaturated fatty acids and their health benefits. *Annual Review of Food Science and Technology*. 2018;**9**:345-381
- [33] Shinn S, Proctor A, Baum J. Egg yolk as means for providing essential and beneficial fatty acids. *Journal of the American Oil Chemists' Society*. 2018;**95**:5-11. DOI: 10.1146/annurev-food-111317-095850
- [34] Kralik G, Kralik Z, Galovic O, Hanžek D. Cholesterol content and fatty acids profile in enriched n-3 PUFA and conventional eggs. In: *World Poultry Congress 2022; Abstracts presented as webinar*. Book of abstract page 57
- [35] Charoensiriwatana W, Srijantr P, Teeyapant P, Wongvilairattana J. Consuming iodine enriched eggs to solve the iodine deficiency endemic for remote areas in Thailand. *Nutrition Journal*. 2010;**9**:68. DOI: 10.1186/1475-2891-9-68
- [36] Bourre JM, Galea F. An important source of omega-3 fatty acids, vitamins D and E, carotenoids, iodine and selenium: A new natural multi-enriched egg. *Journal of Nutrition, Health & Aging*. 2006;**10**:371-376
- [37] Naber EC. Modifying vitamin composition of eggs: A review. *Journal of Applied Poultry Research*. 1993;**2**:385-393
- [38] Oueslati K, Ribeiro B, Chavatte D, Alleno C, Bouvet R. Positive impact of prebiotics and antioxidants on egg quality at the end of the laying hen production cycle. In: *26th World's Poultry Congress, Abstracts selected in 2020*. p. 145
- [39] Nakamura S, Kato A, Kobayashi K. Enhanced antioxidative effect of ovalbumin due to covalent binding of polysaccharides. *Journal of Agricultural and Food Chemistry*. 1992;**40**:2033-2037. DOI: 10.1021/jf00023a001
- [40] Huang X, Tu Z, Xiao H, Wang H, Zhang L. Characteristics and antioxidant activities of ovalbumin glycosylated with different saccharides under heat moisture treatment. *Food Research International*. 2012;**48**:866-872. DOI: 10.1016/j.foodres.2012.06.036
- [41] Chang O, Ha G, Han G. Novel antioxidant peptide derived from the ultrafiltrate of ovomucin hydrolysate. *Journal of Agricultural and Food Chemistry*. 2013;**61**:7294-7300. DOI: 10.1021/jf4013778
- [42] Liu H, Zheng F, Cao Q, Ren B, Zhu L, Striker G, et al. Amelioration of oxidant stress by the defensin lysozyme. *American Journal of Physiology. Endocrinology and Metabolism*. 2006;**290**:E824-E832. DOI: 10.1152/ajpendo.00349.2005
- [43] Sugino H, Ishikawa M, Nitoda T, Koketsu M, Juneja LR, Kim M, et al. Antioxidative activity of egg yolk phospholipids. *Journal of Agricultural*

and Food Chemistry. 1997;**45**:551-554.
DOI: 10.1021/jf960416p

[44] Stahl W, Sies H. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine*. 2003;**24**:345-351. DOI: 10.1016/S0098-2997(03)00030-x

[45] Ma L, Lin XM. Effects of lutein and zeaxanthin on aspects of eye health. *Journal of the Science of Food and Agriculture*. 2010;**90**:2-12. DOI: 10.1002/jsfa.3785

[46] Zhang LX, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/10T1/2 cells: Relationship to their cancer chemopreventive action. *Carcinogenesis*. 1991;**12**:2109-2114. DOI: 10.1093/carcin/12.11.2109

[47] Cuppett SL, Hall CA. Antioxidant activity of the Labiatae. In: Taylor S, editor. *Advances in Food and Nutrition Research*. Vol. 42. Academic Press Inc.; 1998. pp. 245-271. DOI: 10.1016/S1043-4526(08)60097-2. Available from: <https://www.sciencedirect.com/science/article/pii/S1043452608600972>

[48] Brenes A, Roura E. Essential oils in poultry nutrition: Main effects and modes of action. *Animal Feed Science and Technology*. 2010;**158**(1-2):1-14. DOI: 10.1016/j.anifeedsci.2010.03.007

[49] Harahap U, Dalimunthe A, Hertiani T, Mahatir MN, Satria D. Antioxidant and antibacterial activities of ethanol extract of *Vernonia amygdalina* Delile. Leaves. 2021. The International Conference on Chemical Science and Technology (ICCST – 2020) AIP Conf. Proc. 2342, 080011-1-080011-4; DOI: 10.1063/5.0045447

[50] Milos M, Mastelic J, Jerkovic I. Chemical composition and antioxidant effect of glycosidically bound volatile

compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*). *Food Chemistry*. 2000;**71**(1):79-83. DOI: 10.1016/S0308-8146(00)00144-8

[51] Ofongo RTS, Ohimain EI, Iyayi EA. Qualitative and quantitative phytochemical screening of bitter leaf and neem leaves and their potential as antimicrobial growth promoter in poultry feed. *European Journal of Medicinal Plants*. 2021;**32**(4):38-49. DOI: 10.9734/ejmp/2021/v32i430383

[52] Shahidi F, Janitha PK, Wanasundara PD. Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*. 1993;**32**:67-103. DOI: 10.1080/10408399209527581

[53] Hatano T, Edamatsu R, Mori A. Effects of the interaction of tannins with Co-existing substances. VI.: Effects of tannins and related polyphenols on superoxide anion radical, and on 1, 1-Diphenyl-2-picrylhydrazyl radical. *Chemical & Pharmaceutical Bulletin*. 1989;**37**:2016-2021. DOI: 10.1248/cpb.37.2016

[54] Olamilosoye KP, Akomolafe RO, Akinsomisoye OS, Adefisayo MA, Alabi QK. The aqueous extract of *Ocimum gratissimum* leaves ameliorates acetic acid induced colitis via improving antioxidant status and haematological parameters in male Wistar rats. *Egyptian Journal of Basic and Applied Sciences*. 2018;**5**(3):220-227. DOI: 10.1016/j.ejbas.2018.05.006

[55] Oyem JC, Chris-Ozoko LE, Enaohwo MT, Otabor FO, Okudayo VA, Udi OA. Antioxidative properties of *Ocimum gratissimum* alters Lead acetate induced oxidative damage in lymphoid tissues and haematological parameters of adult Wistar rats. *Toxicology Reports*. 2021;**8**:215-222. DOI: 10.1016/j.toxrep.2021.01.003

- [56] Dauksiene A, Klementaviciute J, Gruzauskas R, Klupsaite D, Bartkiene E. Laying hens' production effectiveness increasing and quality improving by including to their diet sustainable plants (*Helianthus tuberosus* L.). In: 26th World's Poultry Congress, abstracts selected in 2020. p. 443
- [57] Abdel-Wareth AAA, Lohakare JD. Productive performance, egg quality, nutrients digestibility, and physiological response of bovans brown hens fed various dietary inclusion levels of peppermint oil. *Animal Feed Science and Technology*. 2020;**267**:114554. DOI: 10.1016/j.anifeedsci.2020.114554
- [58] Vlaicu PA, Panaite TD. Effect of dietary pumpkin (*Cucurbita moschata*) seed meal on layer performance and egg quality characteristics. *Animal Bioscience*. 2022;**35**(2):236-246. DOI: 10.5713/ab.21.0044
- [59] Vakili R, Toroghian M, Torshizi ME. Saffron extract feed improves the antioxidant status of laying hens and the inhibitory effect on cancer cells (PC3 and MCF7) growth. *Veterinary Medicine and Science*. 2022;**8**:2494-2503. DOI: 10.1002/vms3.910
- [60] Omri B, Alloui N, Durazzo A, Lucarini M, Aiello A, Romano R, et al. Egg yolk antioxidants profiles: Effect of diet supplementation with linseeds and tomato-red pepper mixture before and after storage. *Foods*. 2019;**8**:320. DOI: 10.3390/foods8080320
- [61] Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;**181**:1199-1200. DOI: 10.1038/1811199a0
- [62] Steel RGD, Torrie JH. Principles and Procedures of Statistics. A Biometrical Approach. 2nd ed. New York, USA: McGraw-Hill; 1980. pp. 20-90
- [63] Oriakhi K, Oikeh EI, Ezeugwu NO, Anoliefo O, Aguebor OES. Comparative antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves. *Journal of Agricultural Science*. 2014;**6**:13-20. DOI: 10.5539/jas.v6n1p13
- [64] Ekaluo UB, Ikpeme EV, Ekerette EE, Chukwu CI. *In vitro* antioxidant and free radical activity of some Nigerian medicinal plants: Bitter leaf (*Vernonia amygdalina* L.) and Guava (*Psidium guajava* Del.). *Research Journal of Medicinal Plant*. 2015;**9**(5):215-226. DOI: 10.3923/rjmp.2015.215.226
- [65] Bork PM, Schimitz ML, Kuhnt M, Escher C, Heinrich M. Sesquiterpene lactone containing Mexican Indian medicinal plants and pure sesquiterpene lactones as potent inhibitors of transcription factor NF- κ B. *FEBS Letters*. 1997;**402**:85-90. DOI: 10.1016/S0014-5793(96)01502-5
- [66] Haraguchi H, Ishikawa H, Sanchez Y, Ogura T, Kubo Y, Kubo I. Antioxidative constituents of *Heterotheca inuloides*. *Bio-organic & Medicinal Chemistry*. 1997;**5**:865-871. DOI: 10.1016/S0968-0896(97)00029-1
- [67] Jodynis-Liebert J, Murias M, Bloszyk E. Effect of sesquiterpene lactones on antioxidant enzymes and some drug-metabolizing enzymes in rat liver and kidney. *Planta Medica*. 2000;**66**:199-205. DOI: 10.1055/s-2000-8566
- [68] Igile GO, Oleszek W, Jurzysta M, Burda S, Fanfunso M, Fasanmade AA. Flavonoids from *Vernonia amygdalina* and their antioxidant activities. *Journal of Agricultural and Food Chemistry*. 1994;**42**:2445-2448. DOI: 10.1021/jf00047a015
- [69] Erasto P, Grierson DS, Afolayan AJ. Antioxidant constituents in *Vernonia amygdalina* leaves. *Pharmaceutical Biology*. 2007;**45**(3):195-199. DOI: 10.1080/13880200701213070