

**PHYSICOCHEMICAL CHARACTERISTICS
AND ANTIOXIDANT ACTIVITIES OF JAPANESE
QUAIL (*COTURNIX COTURNIX JAPONICA*)
EGG YOLK OIL (QEYO) EXTRACTED USING
TWO DIFFERENT METHODS**

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Abstract

In this study, the physico-chemical characteristics, mineral, phenolic contents and antioxidant activities of QEYO were evaluated. The oil was extracted using gentle heating (GH) and *n*-hexane (NH) methods, extracts were subjected to proximate, physicochemical and mineral analysis using atomic absorption spectrophotometry (AAS). Total phenolic contents (TPC) was quantified using Folin-Ciocalteu method; while the antioxidant activity were measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) – radical scavenging, ferric reducing antioxidant activity (FRAP) and total flavonoid contents. Physicochemical screening showed the presence of saponins, peroxides, ash, moisture and iodine, with little anthraquinones and cardiac glycosides. Sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P), were obtained in both fractions. Oil extracted by GH had the higher TPC and FRAP values ($p < 0.05$), while the NH fraction was higher in (TFC) ($p < 0.05$). QEYO showed antioxidant activities and can be used for the management of different diseases related to oxidative stress.

Introduction

The nutritional, vitamins and minerals content of quail eggs are much higher than those offered by other eggs. They are especially rich in the essential amino acids (methionine, lysine, and phenylalanine), and are rich sources of antioxidants, minerals, vitamins, and nutrients (WUNJUNTUK et al. 2016). The egg have been claimed to improve metabolism, combat stress, helps in the treatment of obesity, asthma and various forms of allergies. (LALWANI 2011, TUNSARINGKARN et al. 2013). Regular consumption of quail eggs helps fight against many diseases as it is a natural combatant against digestive tract disorders such as stomach ulcers, strengthen the immune system, promote memory health increase brain activity and stabilize nervous system (TRUFFIER 1978). Recently, (LESNIEROWSKI and STANGIERSKI 2018) reported that, eggs are not only a highly nutritious food, but also a rich source of diverse bioactive components also including nutraceutical. Quail pickled egg have been reported to offer such nutritious, ready-to-eat product to the consumers in Egypt (BAYOMY et al. 2017). Because of their extraordinary nutritional and medicinal properties, they are being used with more and more success in Europe and America as well as in the far east (DOWARAH and SETHI 2014). Egg yolk oil, also called ovum oil is derived mostly from the yolk of chicken eggs, although it can be derived from goose, duck and other avian eggs. It consists mainly of triglycerides with traces of lecithin, cholesterol, xanthophylls such as lutein and zeaxanthin and immunoglobulins (WALKER and EMERSON 1964). Oil extracted from hens and duck egg has anti-inflammatory and analgesic effects (HANDE 1983). Solvent extraction of egg oil has been reported to be the simplest and inexpensive method of extraction of egg yolk (KOVALCUKS and DUMA 2014a, WU et al. 2016), and the use of hexane in a combination of 70/30 in ratio with 2-propanol tends to produce more oil in term of yield and β -carotene content. Furthermore, hexane has been identified to be the most popular solvent for lipid extraction in food application (KOVALCUKS and DUMA 2014b). On the other-hand, egg oil extracted using the gentle heating method was reported to have the best anti-inflammatory effects (MAHMOUDI et al. 2013). Therefore this study was design to compare the two inexpensive extraction methods of yolk oil from quail egg, with the aim of comparing them in term of yield, mineral and antioxidant content for recommendation for use in cosmeceutical and drug industries.

Materials and Methods

Quail Egg Material

The eggs were purchased from a local market in Abuja Nigeria. They were identified and authenticated by an Avian Pathologist from the Department of Veterinary Pathology, Usmanu Danfodiyo University, Sokoto, Nigeria.

Preparation of Quail egg powder

Two Hundred and forty (240) quail eggs yolk were manually separated from the egg white as described by (MAHMOUDI et al. 2013) sundried in a beaker at 31–37°C and subsequently blended till a powdery form was obtained. Eight hundred gram (800 g) of the powder was divided into two equal parts for the extraction of the oil. The extraction of the Quail egg yolk oil was performed using the *n*-hexane method of extraction (WARREN et al. 1988) and gentle heating method (MAHMOUDI et al. 2013).

Quail Egg yolk oil Extraction using *n*-hexane

The extraction was performed in the Pharmacology and Toxicology Department, Faculty of Pharmaceutical Science, UDUS, using the Soxhlet extractor (Konte, USA). Four hundred grams (400 g) of the powdered egg sample was poured into a porous thimble and placed in a Soxhlet extractor, using 150 cm³ of N-hexane (with boiling point of 40–60°C) as extracting solvent for 6 hours. The oil was obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70°C to remove the excess solvent from the extracted oil. The oil was then stored in freezer at – 2°C for subsequent studies (WARRA et al. 2011b, WARREN et al. 1988).

Gentle heating extraction of Quail Egg yolk oil

Egg yolk oil was prepared from 400 g of egg powder. Yolk powder was directly heated for about 4 hours gently and filtered with cheesecloth size 50, (Vertical x Horizontal thread 28 x 2) cm filter. The filtrate was used as yolk oil prepared by direct heating (MAHMOUDI et al. 2013).

Oil yield (OY) determination [%]

Solvent that was freed from the oil obtained after extraction was placed over a water bath at 70°C for 30 mins and the volume of oil was recorded and expressed as oil content [%] as calculated below:

$$\% \text{ yield} = \left(\text{wt of } \frac{\text{oil}}{\text{wt}} \text{ of egg yolk powder} \right) \cdot 100$$

(PRITCHARD and ROSSELL 1991)

Preliminary physicochemical, proximate and mineral analysis of Quail Egg Yolk Oils Moisture content (MC) determination

Phytochemical screening of Quail Egg Yolk oil extracted through the gentle heating and *n*-Hexane extraction methods was carried out as follows.

A crucible was washed and dried in the oven, after cooling in the desiccator and weighed (W_1). 1.16 g of the sample was carefully weighed in the crucible and the weight was taken as (W_2). The crucible containing the sample was then placed in an oven at temperature of 105°C for 1 hour. It was cooled and weighed. The crucible was then introduced into the oven again and process of cooling and weighing continued at intervals until a constant weight was obtained (W_3) (COX and PEARSON 1962).

Percentage moisture content was calculated using the formula:

$$\% \text{ MC [g]} = \left(W_2 - \frac{W_3}{W_2} - W_1 \right) \cdot 100$$

where:

W_1 – weight of empty crucible [g]

W_2 – weight of crucible with extract [g]

W_3 – weight of crucible after heating [g]

Ash content (AC) determination

A porcelain crucible was washed and dried in an oven then cooled in a desiccator and weighed. 0.53 g of the sample was carefully weighed in the crucible containing the sample and was heated gently on a Bunsen burner until the smoke ceased. It was then transferred to a muffle furnace and heated at a temperature of 550–570°C for 2 hours to burn all organic

matter. The crucible was taken out of the muffle furnace after a white residue was observed and placed in a dessicator to cool and weighed (COX and PEARSON 1962).

$$\% AC[g] = \left(\text{wt of } \frac{\text{ash}}{\text{wt}} \text{ of sample} \right) \cdot 100$$

Relative density (RD) determination

A specific density bottle was washed, dried and weighed (W_1). It was filled with distilled water and weighed (W_2). The water was poured off and the bottle was dried to its previous constant weight and then filled with the oil sample and weighed (W_3) (COX and PEARSON 1962).

$$RD = (W_2 - \frac{W}{W_1} - W_3)$$

Iodine value (IV) determination

Iodine monochloride (13.6 g) was dissolved in 8243 ml glacial acetic acid and cooled. 25 ml of this solution was then titrated against 0.1 N sodium thiosulphate. Another portion of 200 ml of glacial acetic acid was added to 3ml of bromide, and 5 ml of this solution was added to 10 ml of 15% potassium iodide solution and titrated against 0.1 N sodium thiosulphate and mixed well.

Oil samples (0.5 g) was weighed into an iodine flask and dissolved in 10 mL of chloroform this was followed by 25 ml of Hanus solution. The flask was stopped and kept in the dark cup board for 30 minutes at room temperature; 10 ml of 10% of potassium iodide solution and 100 ml of freshly boiled and cooled water added. This was titrated against 0.1 ml Sodium thiosulfate solution using starch as an indicator. A blank titration was also conducted under the same conditions without the sample (COX and PEARSON 1962).

$$IV = \left(\frac{(B - S) \cdot N \cdot 12.69}{g} \right) \text{ of sample}$$

where:

B – mL thiosulphate for blank

S – mL thiosulphate for the sample

N – normality of thiosulphate solution.

Peroxide value (PV) determination

One gram (1 g) of the oil sample was transferred into 20 ml flask and 1 g of powdered potassium iodide (KI) and a solvent mixture (2:1 of glacial acetic and trichloromethane) were then added. The solution was then placed on a water bath for a few minutes for complete dissolution. 20 ml of 50% potassium iodide was then introduced into the conical flask and the sample titrated with 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$. The indicator was a regular starch solution. Blank experiment was similarly performed (COX and PEARSON 1962).

$$\text{PV} = \left(\frac{S \cdot N}{g} \text{ sample} \right) \cdot 100$$

where:

S – mL $\text{Na}_2\text{S}_2\text{O}_3$ Test–Blank)

N – normality of $\text{Na}_2\text{S}_2\text{O}_3$.

Saponification value (SV) determination

The oil sample at 5.0 g was weighed in to 200 ml conical flask and 25 ml of 0.5 M of ethanolic potassium hydroxide solution was added. The flask was configured to a condensing set-up and heated on a water-bath for 1 hour with frequent shaking and the content was allowed to cool. The solution was then titrated with warm 0.5 M hydrochloric acid using 1% phenolphthalein indicator. PEARSON 1991, Equivalent titration was performed for the blank and generated values were employed for computation according to the following relation

$$\text{SV} = A - B \cdot 28.05/Q$$

where:

A – volume of 0.5 M of hydrochloric acid used in the blank titration,

B – volume of 0.5 M of hydrochloric acid used in the sample titration,

Q – weight in grams of the oil sample 28.05 = conversion factor

Cardiac Glycosides (PG)

One mL of the sample was extracted with 10 cm³ of 70% ethanol and filtered. From the filtrate, 8 cm³ was transferred into 100 cm³ volumetric flask and the volume made to the mark with distilled water and filtrate. 8 cm³ of 12.5% lead acetate solution was added to 8 cm³ of the filtrate. The

mixture was shaken thoroughly and transferred into a 100 cm³ volumetric flask and made to the mark with distilled water and filtered. A 50 cm³ of the filtrate was then pipetted into 100 cm³ flask and 8 cm³ of 4.5% Na₂HPO₄ solution and added. This mixture was filtered twice and 10 cm³ of Baljet's reagent was added to the filtrate.

A blank experiment was also prepared by excluding the sample from the mixture, but all other conditions were kept constant. The mixture was allowed to stand for an hour for the colour to develop and the intensity of the colour was measured using Spectrophotometer at 495 nm (COX and PEARSON 1962). Percentage of glycoside was calculated as follows:

$$\text{PG [\%]} = A \cdot 100/70$$

Anthraquinones

Two mL of the oil extract was boiled with 5 ml of 10% hydrochloric acid for 3 minutes. 5 ml of chloroform was added. 5 drops of 10% ammonia was further added. A rose pink coloration indicates a positive result (HARBORNE 1998). Absorbance of the resulting colour was then measured using 1 cm cuvette.

Determination of Mineral Elements by Wet Digest Method

The elements were extracted from the oil by the wet digest method (TAIYE and ASIBEY-BERKO 2001). The digested sample was analysed for the elemental composition using Atomic Absorption Spectrophotometer (AAS). Na, K, Mg, and Ca were determined and the concentrations of the elements were presented in mgL⁻¹.

Determination of effect of oil extract On 2,2-diphenyl-1 picryl hydrazyl (DPPH) radical

The extract (0.02 g) was dissolved in 20 mls of 95% methanol and stirred using a stirrer, then 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1.0 ml from mixture was poured into 5-sets of test tubes labelled (*A* to *E*) and one test tube was used as control which contains 1.0 ml of distilled water labelled (*F*). 1 ml of DPPH reagent was added into each of test tubes labelled (*A* to *F*). The absorbance of tests and that of control were measured spectrophotometrically at 517 wavelength (HATANO et al. 1998)

Estimation of total phenolic content using Folin–Ciocalteu's reagent

The extract (0.1 g) was dissolved in 50 ml of distilled water after which solution was mixed, 2 mls of mixture was poured into 3 sets of test tubes. 2 mls of 17% Na_2CO_3 into each of test tube. One test tube was used as control. Then 0.5 ml of Folin–Ciocalteu's reagent was added into test tube after which the sample were centrifuged using centrifuge machine at 4,000 revolution/minute. The supernatant was collected in fresh 2 ml micro-tubes absorbance of test and controlled was measured at 765 nm wavelength.

Determination of ferric reducing antioxidants assay (FRAP)

The ferric reducing antioxidant power of the oil extract was determined in this study by reacting the extract (1 ml) with (5 ml) of distilled water, then 1 M HCl (1.5 ml) and 1% potassium ferricyanide (1.5 ml) was added. Then sodium dodecyl sulphate (SDS, 0.5 ml) and 0.5 ml of 0.2% ferric chloride (FeCl_3) were added, and the entire solution was incubated at 50 for 20 min to complete the reaction. Gallic acid was used as the standard and the absorbance was measured using a spectrophotometer at 750 nm, the results were expressed as mg GAE/g d.w. sample.

Determination of flavonoids using precipitation method

A weighed sample of 5 grams quail egg oil was hydrolysed by boiling in 100 mls of hydrochloric acid solution for about 35 minutes. The hydro-lystate was filtered to recover the extract (filtrate). The filtrate was treated with ethyl acetate drop wise twice until in excess. The precipitated flavonoid was recovered by filtration using a weighed filter paper after drying in an oven at 100°C for 30 minutes; it was cooled in a desiccator and reweighed. The difference in weighed gave the weighed flavonoid which was expressed as a percentage of the weighed of sample analysed.

Statistical Analysis

The data obtained from the study were analysed using INVIVOSAT statistical software and data was expressed as mean \pm standard error of mean (SEM), while significant difference was determine using Student *T*-Test, and values less than 0.05 were considered to be statistically significant.

Results

Preliminary Physicochemical Properties of QEO

The results of the preliminary physicochemical screening show the presence of saponins, peroxides, ash, moisture and iodine, with very small amounts of anthraquinones and cardiac glycosides. The saponins, and Iodine content are relatively higher in the oil extracted using the gentle heating method, while the peroxide value, moisture content and the yield of the oil sample is higher in oil extracted using the *n*-hexane solvent as shown in Table 1.

Table 1

Preliminary physicochemical screening results of QEO

QEO/Parameter	QEO (GH)	QEO (NH)
Saponification value [mg KOH/g]	237.9*	230.5
Peroxide value [mq H ₂ O ₂]	7.0	10.0*
Iodine value [gI ₂ /100 g]	21.83*	18.82
Ash content [%]	1.89	1.42
Moisture content [%]	13.04	19.83*
Cardiac glycoside [g/100 g]	0.093	0.078
Oil yield [%]	15.9	35*
Relative density [g]	0.84	0.83
Anthraquinone [mg g ⁻¹]	0.012	0.015

QEO – quail egg yolk oil NH – *N*-Hexane GH – gentle heating

* Values significantly higher when compared between the two extraction methods ($p < 0.05$)

Mineral Content of QEO

The results for the preliminary mineral screening shows the presence of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P), in the quail egg yolk oil for both *n*-Hexane and gentle heating extraction processes. Calcium and potassium level are higher in oil extracted using the gentle heating method while sodium, magnesium and phosphorus level are higher in the oil extracted using the *n*-hexane solvent as shown in Table 2.

Table 2

Preliminary mineral screening results of QEO

QEO/parameter [mg L ⁻¹]	Na	K	Ca	Mg	P
QEO(GH) [mg L ⁻¹]	12.5	130.0*	0.90*	0.60	4.65
QEO(NH) [mg L ⁻¹]	25.0*	92.5	0.55	1.95*	6.41*

Note: QEOGH – quail egg yolk oil gentle heating; QEONH – quail egg yolk oil; NH – N-Hexane; Na – sodium; K – potassium; Ca – calcium; Mg – magnesium; P – phosphorus

* Values are significantly higher when comparing the values between the two extraction methods ($p < 0.05$)

Total phenolic content (TPC)

2,2-diphenyl-1,1-picrylhydrazyl (DPPH)

The TPC results showed that, quail egg samples extracted using the gentle heat method showed higher total phenolic content with high absorbance level compared to the egg samples extracted using the *n*-hexane extraction ($P > 0.05$) as shown in Figure 1.

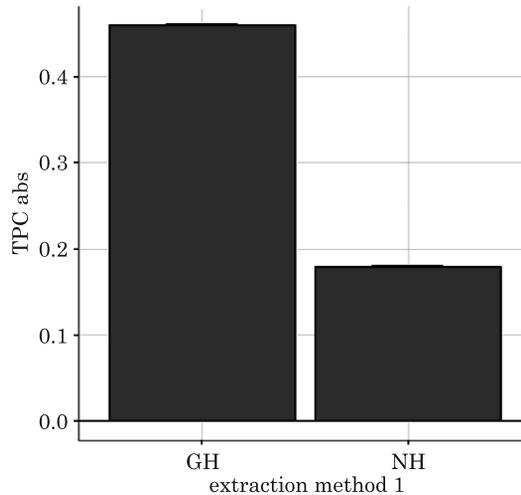


Fig. 1. Showing the mean \pm SEM total phenolic content of quail egg oil extracted using the gentle heat and *n*-hexane methods;

* Indicate a significant difference between the two methods of extraction ($p < 0.05$)

The DPPH antioxidant results between the two group shows that, DPPH scavenging activities is higher in egg samples extracted using the gentle heat method compared to *n*-hexane extraction method although not significant ($P > 0.05$) as shown in Figure 2.

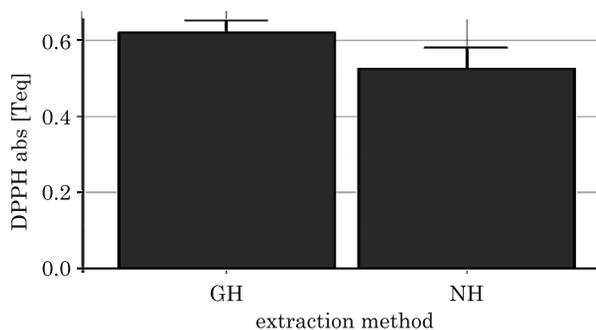


Fig. 2. Showing mean \pm SEM DPPH scavenging activity using gentle heat and *n*-hexane extraction methods. No significant difference when comparing the absorbance level between the two methods of extraction ($p > 0.05$)

Ferric reducing antioxidants power (FRAP)

The Ferric reducing antioxidant results between the two group shows that, FRAP is higher in egg samples extracted using the gentle heat method compared to *n*-hexane extraction method. ($P > 0.05$) as shown in Figure 3.

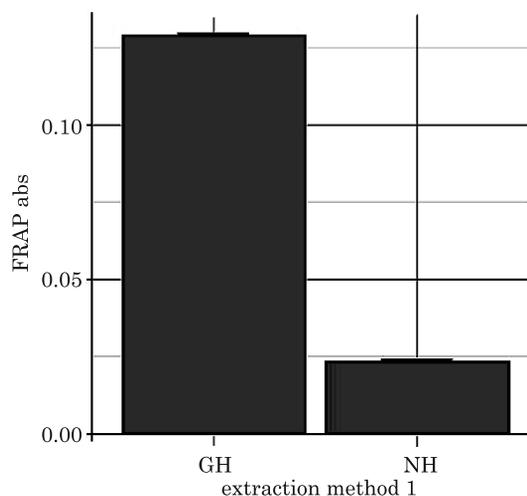


Fig. 3. Showing the mean \pm SEM Ferric reducing antioxidants property of quail egg oil extracted using the gentle heat and *n*-hexane method of extraction

* Indicate a significant difference between the two methods of extraction ($p > 0.05$).

Total flavonoid contents (TFC)

The TFC results showed that, quail egg samples extracted using the gentle heat method showed lower total flavonoid content with low absorbance level compared to the egg samples extracted using the *n*-hexane extraction ($P > 0.05$) as shown in Figure 4.

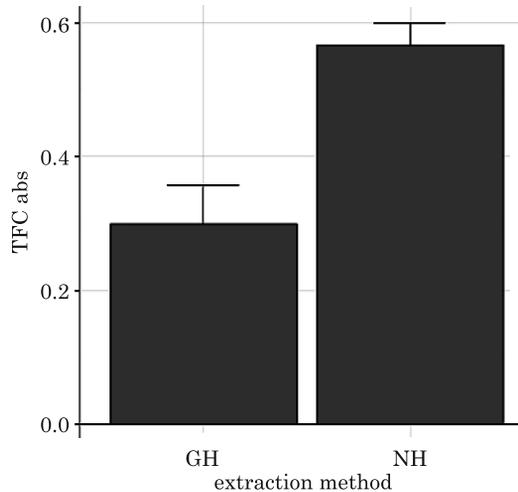


Fig. 4. Showing the mean \pm SEM total flavonoid content of quail egg oil extracted using the gentle heat and *n*-hexane methods of extraction;

* Indicate a significant difference between the two methods of extraction ($p < 0.05$)

Discussion

Oils are known for their use in various medicinal purposes and sometime as adjuvants or co-therapeutic agents. Extraction of oil from quail egg and the quantification of its bioactive compounds with the study of its antioxidant properties have not been reported in the literature. The percentage yield of crude oil obtained in this study was higher in the fraction extracted using *n*-hexane (35%), previous study by (LARSEN and FRONING 1981) also reported a high yield of the oil using *n*-hexane. KOVALCUKS and DUMA 2014a reported a yield of $28.90 \pm 0.27\%$ and $26.37 \pm 1.04\%$ with 2-propanol/hexane and ethanol/chloroform solvent combination. Although, the *n*-hexane solvent extraction gives the high yield than the gentle heating method (15.9%), previous research reported high concentration of residual solvents which limits the usage of egg oil which were extracted using solvents in food or in cosmetics (KOVALCUKS 2014). Preliminary mineral element screening of Quail egg yolk oil (QEYO) extracted with the gentle

heating and *N*-Hexane methods of extraction revealed the presence of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and phosphorus (P). A significant concentrations of potassium was obtained in the two oil extracts (130 mg L⁻¹ for GH fraction and 93 m L⁻¹ for the NH extract) which was higher when compared to what was obtained in raw quail egg (63.0 mg L⁻¹) by (OLUWAFEMI and UDEH 2016) . The preliminary physico-chemical analysis showed the presence of saponins in the oil. QEOGH and QEONH had an abundance of saponins with 237.90 mg KOH g⁻¹ and 230.57 mg KOH g⁻¹ respectively. Saponification value of cotton seed was estimated to be 199.42 ± 0.53 mg KOH g⁻¹ while neem seed oil is 213 mg KOH g⁻¹, groundnut oil is 168 mg KOH g⁻¹ while moringa oil is 155.68 mg KOH g⁻¹ , coconut oil however has a higher saponins content of 253.2 mg KOH g⁻¹ (AFOLAYAN et al. 2014, WARRA et al. 2011a).

Anthraquinone content of QEO was 0.0012 mg in QEOGH and 0.0015 mg in QEO (NH). Anthraquinones are known to have anti-inflammatory and purgative and emetic effects. They also possess antihypertensive effects and have been shown to inhibit angiotensin converting enzyme (ACE) in rats at a dose of 169.93 microgram L⁻¹ (HYUN et al. 2009).

Total phenolic contents and antioxidants activity of quail egg oil was best demonstrated in the samples extracted using the gentle heating method therefore, the findings of the present study revealed that extraction method plays an important role in determining the total phenolic contents and antioxidant activity of quail egg oil. Extraction methods has a tremendous effect on the yield of bioactive compounds and antioxidant activities of some products (SANI et al. 2012).

The gentle heat method used in this work has higher total phenolic content relatively compared to *n*-hexane method of oil extraction; the result is in accordance with previous studies which shows that efficiency antioxidants phenolics is lowest in hexane extracts of cashew shoot (RAZALI et al. 2008). Similarly, the result shows that quail egg oil extracted using gentle heat method has higher antioxidant activity for DPPH and FRAP antioxidants relatively compared to *n*-hexane method of extraction, the anti-inflammatory effects observed in the previous studies using the gentle heating extraction method might be linked to the presence of these antioxidants. Previous research showed that, hexane extract of *Limonium delicatulum* had the lowest anti-radical activity while ethanol had the highest (MEDINI et al. 2014). This might be the reason why gentle heat oil extract had higher DPPH and FRAP antioxidant activities compared to oil extract of *n*-hexane.

However, present study revealed that *n*-hexane method of extraction had higher total flavonoid content compared to gentle heat method of

extraction. Drastic reduction in flavonoid content of broccoli-based bars treated with heat was reported by (BARAKAT and ROHN 2014).

The DPPH antioxidant potential of quail egg oil extracted using gentle heating method of extraction is higher than *n*-hexane method but was not statistically significant ($p > 0.05$). The result showed that the total phenolic content and ferric reducing antioxidants activity were significantly higher ($P < 0.05$) in quail egg oil extracted using gentle heat method but statistically, quail egg oil using *n*-hexane extraction method has significantly higher total flavonoid content relatively compared to gentle heat method of extraction. The higher antioxidant potential of quail egg oil obtained in this study was not surprising due to the higher content of vitamin E (tocopherol, 5920.0 $\mu\text{g } 100 \text{ g}^{-1}$) obtained in the raw yolk reported by (TUNSA-RINGKARN et al. 2013).

Conclusion

The results of the present study showed that, *n*-hexane have the higher yield in term of extraction, but the fraction of the oil extracted using the gentle heating method portrays a higher antioxidants and other chemical constituents, and this might be the reasons for its reported anti-inflammatory effect. High yield of saponins, anthraquinones and other bioactives which are higher compared to those extracted in other products gives the quail egg an upper hand to be utilized as source of antioxidant and other bioactives for further utilization in cosmeceutical and pharmaceutical industries.

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