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Qualitative Phytochemical Screening, Lipoidal Matter and Proximate Analysis of *Codiaeum variegatum* cv. Gold Dust Leaves and Stems Cultivated in Egypt

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ABSTRACT

Objectives: The current study aimed at the qualitative phytochemical screening, lipoidal matter investigation and proximate analysis of *Codiaeum variegatum* cv. Gold Dust (family Euphorbiaceae) leaves and stems for the first time. **Methods:** Phytochemical screening was accomplished using the standard adopted procedures as per constituent. Saponifiable and unsaponifiable components were analyzed using gas liquid chromatography coupled with flame ionization detector (GLC/FID). Finally, the Association of Official Analytical Chemists (AOAC) standard procedures were applied for the proximate analysis of crude fiber, fat, moisture and ash contents. **Results:** The qualitative phytochemical screening revealed the presence of flavonoids in all tested fractions, while anthraquinones and coumarins were completely absent. In addition, the residue was dedicated for the presence of various secondary metabolite classes as; alkaloids and/or nitrogenous bases, carbohydrates, cardiac glycosides, saponins, and tannins. On the other hand, dichloromethane soluble fraction demonstrated the presence of terpenes and/or sterols which were quantitatively identified using gas chromatography (GC) analysis. The GLC of lipoidal matter tentatively revealed the identification of 9-octadecenoic (oleic acid) as the major unsaturated fatty acid (27.90%), while the hexadecanoic (palmitic acid, 18.68%) was pointed out as the most abundant, saturated one. Furthermore, *n*-hexacosane (16.45%), β -sitosterol (1.40%), and α -amyrin (1.90%) represented the major, identified saturated hydrocarbon, sterol, and triterpene, respectively. Finally, the proximate analysis was denoted by the high crude fiber content (21.70 %), moderate ash (14.91%) and moisture (10.77%) values, while the fat content was relatively low (1.00%). **Conclusion:** From the eventual findings it could be concluded that *Codiaeum variegatum* cv. Gold Dust leaves and stems are valuable source of a variety of bioactive secondary metabolites, crude fibers, unsaturated fatty acids and sterols that may serve as bioactive dietary supplement, but it still needs further safety studies.

Keywords: *Codiaeum variegatum* cv. Gold Dust; Lipoidal matter; Phytochemical screening; Proximate analysis

INTRODUCTION

Plants have evolved and adapted over millions of years to produce unique, structurally diverse secondary, bioactive metabolites¹. A knowledge which

promoted the screening of medicinal plants as potential hits and the isolation of many other natural products that may become a known pharmaceutical. In a plant-based screening programs, qualitative and quantitative methods are usually applied to an initial plant or

plant-extract's sample to evaluate their phytochemical profile and hence, predicting their possible medicinal value.

The qualitative, phytochemical method refers to the qualitative detection of the plant's primary and secondary metabolites such as carbohydrates, tannins, flavonoids, anthraquinones, saponins, coumarins, sterols, triterpenes, and alkaloids using a well-known, standard chemical procedure²⁻¹². On the other hand, quantitative analysis refers to the determination of the phytoconstituents, fat, minerals, fiber, and moisture percentage in certain plant sample expressed in terms of mass, concentration, or relative abundance^{13, 14}.

Codiaeum variegatum L. (family Euphorbiaceae), commonly known as garden or variegated croton, is an ornamental shrub with more than 200 varieties and cultivars existing in the ornamental horticulture. They are available in different leaves size, shape, and color patterns¹⁵. The observed health benefits of different cultivars of *C. variegatum* as cytotoxic, antioxidant, anti-inflammatory and antifungal activities may be credited to various reported phytochemicals, examples as but not limited to; phenolic acids, flavonoids, and alkaloids¹⁶⁻¹⁹. Among the unexplored cultivars is the Gold Dust, which is a compact indoor plant that it is characterized by having elliptical green leaves liberally dusted with yellow specks, given this cultivar its common name; the Gold Dust²⁰. However, to date, no qualitative or quantitative analysis has been reported on the cultivar under investigation, hence inspire the lunch of the current study.

The study herein, aimed at the qualitative and quantitative investigation of *Codiaeum variegatum* cv. Gold Dust for the first time. The qualitative phytochemical standard procedures were adopted to detect the presence of various bioactive metabolites, while gas-liquid chromatography and proximate analysis were assumed to execute the quantitative investigation of the lipoidal matter, total ash, moisture, fat, and fiber contents.

MATERIAL AND METHODS

Plant material

Codiaeum variegatum L. cv. Gold Dust leaves and stems were collected at Al-Zohriya Garden, Giza, Egypt, during December 2014. A plant sample was kindly identified by Dr. Thérèse Labib, Head of the Taxonomists at Al-Orman Botanical Garden Herbarium, Giza, Egypt. A voucher specimen (01Cva/2014) was deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt.

Chemicals

Concentrated NH₄OH, α -naphthol, conc. H₂SO₄, AlCl₃, Mg-turnings, conc. HCl, FeCl₃, picric acid, NaOH, acetic anhydride, glacial acetic acid, CH₂Cl₂, CHCl₃, NaOH, Cu-acetate, KI, tartaric acid, bismuth subnitrate, HgCl₂, CH₃OH, butanol, ethyl-acetate were supplied by the Faculty of Pharmacy, Helwan University for the phytochemical screening. Petroleum ether, KOH, diethyl ether, conc. HCl, conc. H₂SO₄, CH₃OH, diazomethane, anhydrous Na₂SO₄, CHCl₃ were supplied by the Cairo University Research Park (CURP, Faculty of Agriculture, Cairo University, Giza, Egypt) and used for the proximate analysis. All authentic references of hydrocarbons, sterols and fatty acids were supplied by the National Research Center (NRC, Cairo, Egypt) for the lipoidal matter analysis.

Methods

Phytochemical screening

The air, dried powdered leaves and stems of *Codiaeum variegatum* cv. Gold Dust (100 g) were extracted with 80% aqueous MeOH followed by successive fractionation using dichloromethane, ethyl acetate and *n*-butanol to obtain DCM, EtOAc, BuOH soluble fractions and a residue. A sample of each fraction and the residue were subjected to qualitative phytochemical screening following the standard procedure per each constituent.

Alkaloids and/or nitrogenous bases²

A dried sample of each fraction was dissolved in 5 ml 1% HCl, then screened for the presence of alkaloids and/or nitrogenous bases using Mayer's and Dragendorff's reagents.

Mayer's test

About 2 ml of Mayer's reagent (K₂HgI₄ solution) was added dropwise to 2 ml of the prepared acidic solution. The subsequent formation of whitish or creamy precipitate indicates the presence of alkaloids and/or nitrogenous bases.

Dragendorff's test

Few drops of Dragendorff's reagent (KBiI₄ solution) was added to 2 ml of the acidic solution. The development of an orange-red precipitate indicates the presence of alkaloids and/or nitrogenous bases.

Anthraquinones^{3,4}

A dried sample of each fraction was dissolved in 5 ml CHCl₃ or 5 ml 1% HCl (with heating for 5 min) and screened for the presence of free or combined anthraquinones, respectively.

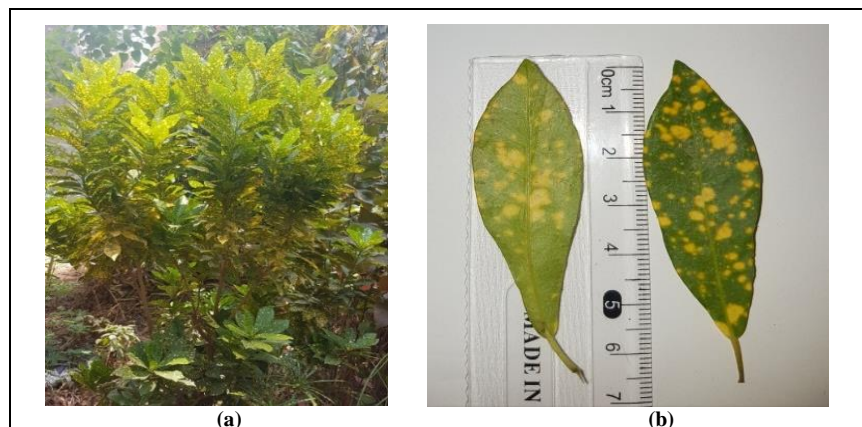
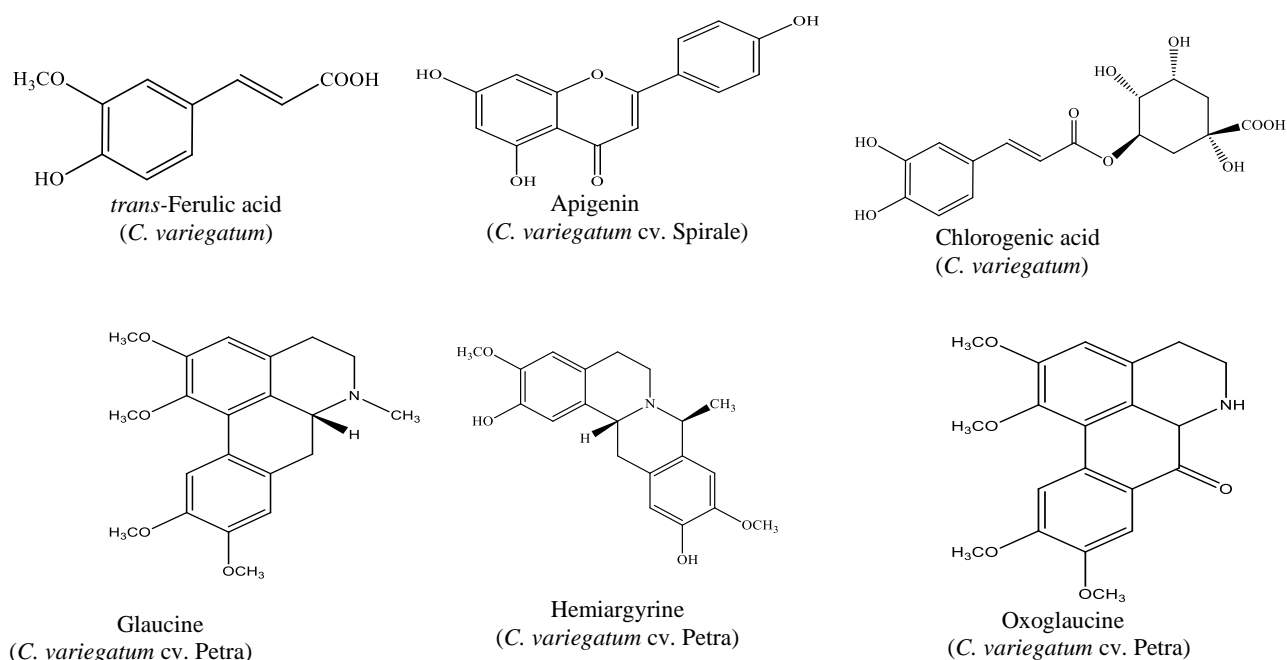


Figure 1. *Codiaenum variegatum* cv. Gold Dust (a) shrub (x 0.12), (b) leaf (x 0.11)



Representative secondary metabolites previously reported from various *Codiaenum variegatum* cultivars

Borntrager's test

About 2 ml of the CHCl₃ extract was shaken with equivalent amount of dil. NH₄OH. The development of rose red color in the aqueous ammonia layer indicates the presence of free anthraquinones.

Modified Borntrager's test

Test for anthraquinones-O-glycoside

Approximately 2 ml of the filtered 1% HCl extract was shaken with an equal volume of CHCl₃. Then, the CHCl₃ layer was separated into another test

tube and shaken with 2 ml dil. NH₄OH. The development of rose red color in the ammonia layer indicates the presence of anthraquinones-O-glycoside.

Test for anthraquinones-C-glycoside

To 2 ml of the filtered 1% HCl extract, 2 ml of 5% aq. FeCl₃ was added, then the solution was boiled for 5 min. The hot solution was filtered, cooled, then shaken with an equal volume of CHCl₃. The CHCl₃ layer was separated into another test tube and mixed with 2 ml of dil. NH₄OH. The development of rose red

color in the ammonia layer indicates the presence of anthraquinones-C-glycoside.

Carbohydrates⁵

A sample of each fraction was dissolved in 5 ml distilled H₂O, then filtered. The filtrate was screened for the presence of carbohydrates using two chemical tests as following:

Molisch's test

To 2 ml of the filtrate, about 2 drops of freshly prepared 10 % alc. α -naphthol were added, then mixed thoroughly. About 2 ml of conc. H₂SO₄ was allowed to flow down the side of the test tube. The appearance of a red or reddish violet ring at the junction of the two layers indicates the general presence of carbohydrates.

Barafoed's test

To 1 ml of the filtrate, was added 1 ml of Barafoed's reagent (Cu-acetate in 1% acetic acid), then the mixture was heated on a water bath for 2 min. The development of a red precipitate of cuprous oxide indicates the presence of monosaccharides.

Cardiac glycosides⁶

Baljet's test

A sample of each fraction was dissolved in 50% aq. MeOH, shaken, then filtered. The filtrate was mixed with an equal volume of freshly prepared Baljet's reagent (1 ml dil. NaOH and 9 ml 1% picric acid). The development of an orange color indicates the presence of cardiac glycosides. Blank experiment should be performed by adding the same volume of 50% aq. MeOH instead of the plant extract, along with the same volume of Baljet's reagent.

Keller-Killiani's test

A sample of each fraction was dissolved in 1 ml glacial. AcOH containing 1 drop of 5% aq. FeCl₃. This was then underlayer with 1 ml of conc. H₂SO₄. A brown ring obtained at the interface of the two layers, indicates the presence of cardiac glycosides.

Coumarins⁷

A sample of each fraction was solubilized in MeOH then, the test tube was covered with a clean filter paper moistened with dil. NaOH. The covered test tube is then placed in a boiling water bath for 10 min. The filter paper was removed and examined under UV (254 and 365 nm). The visualization of a yellow green fluorescence spot on the filter paper indicates the presence of coumarins.

Flavonoids⁸

A sample of each fraction was dissolved in 10 ml of 1% HCl and heated in a water bath for 5 min. The

solution was filtered, divided into 2-portions, then screened for the presence of flavonoids as stated below.

Alkaline reagent test

To 5 ml of the filtrate, was added 5 ml dil. NH₄OH and 1 ml conc. H₂SO₄. The appearance of yellow coloration that disappears on standing indicates the presence of flavonoids.

Aluminum chloride test

Few drops of 1% AlCl₃ were added to 3 ml of the filtrate. The development of a yellow coloration indicates the presence of flavonoids.

Shinoda's test

A sample of each fraction was dissolved in MeOH, then few drops of conc. HCl were added followed by the addition of freshly cut metallic magnesium turning cautiously in small portions. The development of red to orange red colour indicated the presence of flavonoids.

Saponins^{4,9}

Frothing test (Foaming test)

A sample of each fraction was dissolved in dist. H₂O and shaken vigorously for 2 min. The formation of a stable froth was taken as a preliminary evidence for the presence of saponins.

Hemolysis test

A sample of each fraction, dissolved in 1% normal saline, was mixed with 0.2 ml of 10% blood in normal saline. The development of red supernatant compared to a reference control indicates the presence of saponins. In the preparation of the reference control, 0.2 ml of normal saline was added instead of the plant sample.

Tannins^{9,10}

To a sample of each fraction, dissolved in dist. H₂O, few drops of 0.1% FeCl₃ were added gradually. The formation of a brownish-green or a bluish-black coloration indicates the presence of condensed or hydrolysable tannins, respectively.

Terpenoids and/or Steroids¹¹

A sample of each fraction was dissolved in 5 ml CHCl₃, then filtered and the filtrate was subjected to the following chemical tests.

Liebermann-Burchard's test

About 2 ml of the filtrate was transferred to a dry test tube. 2 ml of acetic anhydride and 1 ml of conc. H₂SO₄ were added sequentially. The development of a greenish color which turns blue on standing indicates the presence of steroids.

Table 1. Phytochemical screening of DCM, EtOAc, BuOH soluble fractions and the residue of *Codiaeum variegatum* cv. Gold Dust leaves and stems

Constituents	Chemical test	DCM	EtOAc	BuOH	Residue
Alkaloids and/or nitrogenous bases	Mayer	-	-	-	+
	Dragendorff	-	-	-	+
Anthraquinones					
	a) Free	-	-	-	-
b) Combined	Modified Borntrager	-	-	-	-
Carbohydrates	Molish	-	-	-	+
	Barafoed's	-	-	-	+
Cardiac glycosides	Baljet	-	+	-	+
	Keller-Killiani	-	+	-	+
Coumarins	UV-spot visualization	-	-	-	-
Flavonoids	Alkaline reagent	+	+	+	+
	Aluminum chloride	+	+	+	+
	Shinoda	+	+	+	+
Saponins	Frothing	-	-	-	+
	Hemolysis	-	-	-	+
Tannins	FeCl ₃	-	-	-	+
Terpenoids and/or Steroids	Liebermann-Burchard	+	-	-	-
	Salkowski	+	-	-	-

DCM (Dichloromethane soluble fraction), EtOAc (Ethyl acetate soluble fraction) and BuOH (Butanol soluble fraction)
Qualitative result (+) = present, (-) = absent.

Salkowski's test

To about 2 ml of the filtrate in a dry test tube, was added gradually 3 ml of conc. H₂SO₄ till the formation of a bottom layer. The development of a reddish-brown coloration at the interface of the two-layers indicates the presence of terpenoids.

Investigation of lipoidal matter

Preparation of lipoidal matter²¹

The air-dried, powdered leaves and stems of *Codiaeum variegatum* cv. Gold Dust (20 g) were extracted with petroleum ether (60-80 °C, 5 x 300 ml). The extracts were filtered, pooled together, then evaporated under reduced pressure at 50 °C yielding a viscous residue of 200 mg which was kept in the dark, at -20 °C for further analysis.

Saponification and fractionation of lipoidal matter²²

The petroleum ether extract was saponified with 50 ml of 10% alc. KOH under reflux followed by evaporation of the alcohol, while the remaining aqueous solution was re-extracted with petroleum ether. The collected ethereal extracts were washed with distilled water till completely free from alkalinity (checked by litmus paper), then dried over anhydrous sodium sulphate. The collected filtrate was evaporated under reduced pressure to dryness, weighed, and kept in the

dark at -20 °C for further study of the unsaponifiable matter (USM) by GLC.

The remaining, alkaline aqueous layer left after extraction of USM was transferred to a separating funnel and acidified with 10% HCl to liberate the free fatty acids. The released fatty acids were extracted with pet. ether, in each time the ethereal layer was separated. The collected ethereal extracts were washed with distilled water, dried over anhydrous sodium sulfate, and evaporated to dryness under reduced pressure. The residue that represents the saponifiable matter (total fatty acids, TFA), was weighed and kept in the dark at -20 °C for the derivatization protocol.

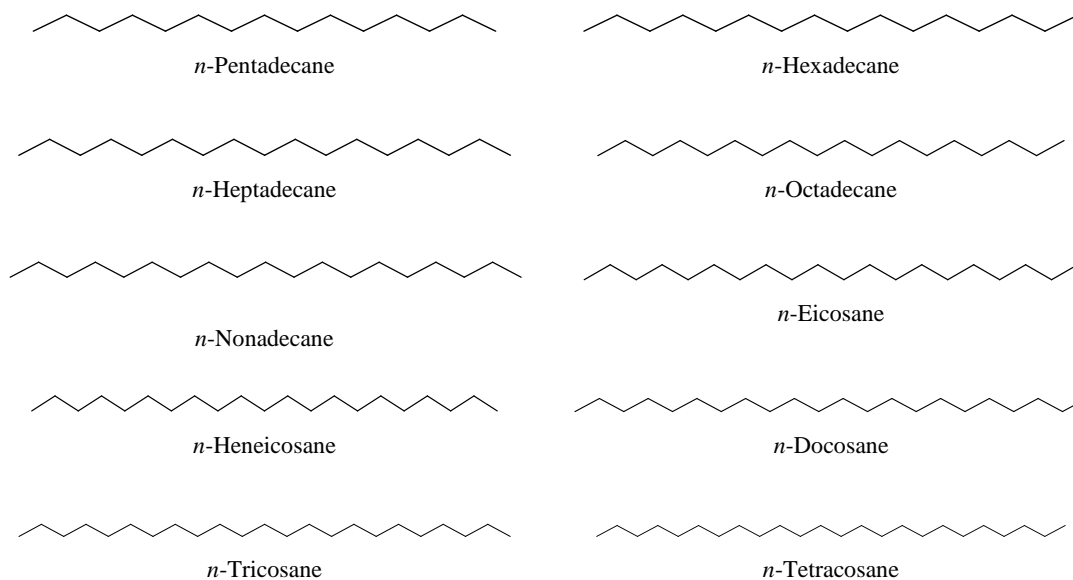
Derivatization of fatty acids to their corresponding methyl esters (FAME)²³

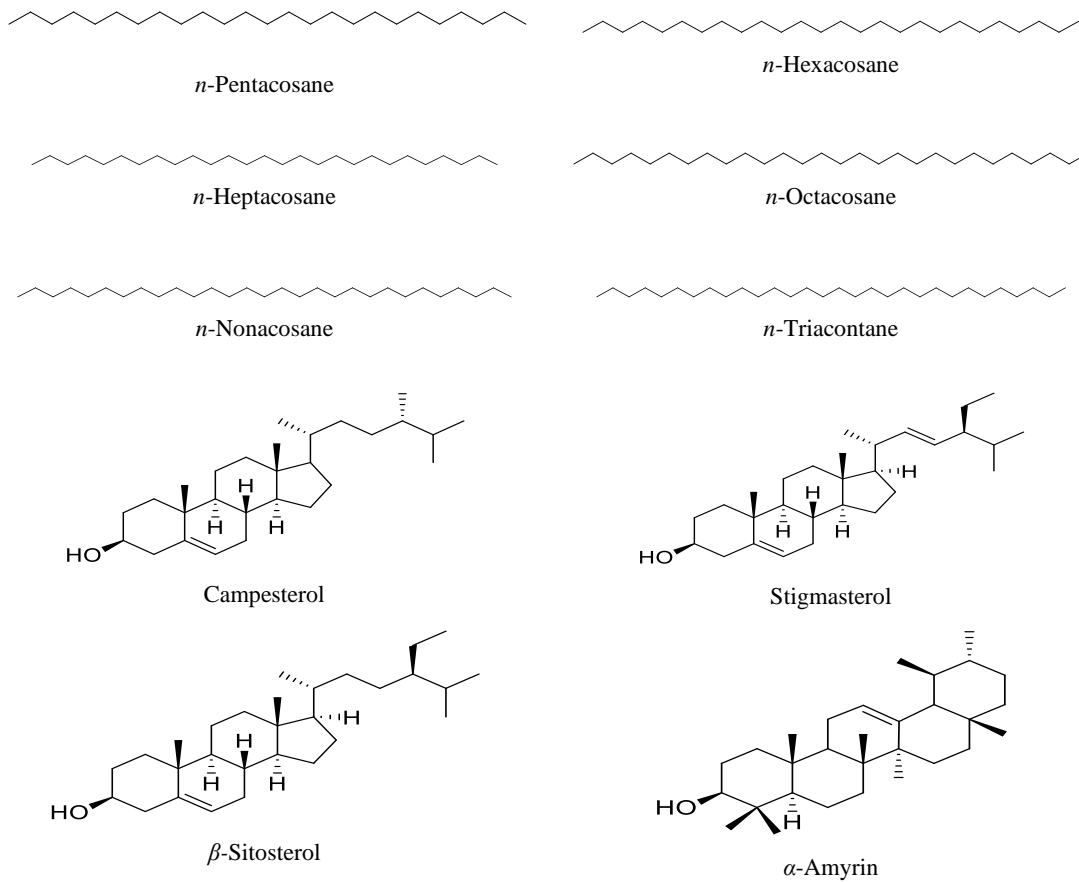
The fatty acids dried residue was dissolved in least amount of anhydrous methanol, then an ethereal solution of diazomethane was added portion wise until gas evolution ceased. The mixture acquired a pale yellow colour indicating the addition of excess of diazomethane. The reaction mixture was left for 10 min at room temperature then, the ether was evaporated under nitrogen stream at room temperature. Two drops of re-distilled chloroform solution were added to dissolve the fatty acids methyl esters (FAME) and 10 ml of this solution were taken for further investigation by GLC.

Table 2. GLC analysis of USM of *Codiaeum variegatum* cv. Gold Dust

RT	Peak area (%)	Identified HC/sterol	Molecular formula	RRT*
11.22	0.37	<i>n</i> -Pentadecane	C ₁₅ H ₃₂	0.46
12.33	0.24	<i>n</i> -Hexadecane	C ₁₆ H ₃₄	0.50
13.05	0.56	<i>n</i> -Heptadecane	C ₁₇ H ₃₆	0.56
14.47	0.86	<i>n</i> -Octadecane	C ₁₈ H ₃₈	0.59
16.24	2.05	<i>n</i> -Nonadecane	C ₁₉ H ₄₀	0.66
17.76	2.42	<i>n</i> -Eicosane	C ₂₀ H ₄₂	0.72
19.71	1.09	<i>n</i> -Heneicosane	C ₂₁ H ₄₄	0.80
20.21	1.5	<i>n</i> -Docosane	C ₂₂ H ₄₆	0.82
21.70	2.26	<i>n</i> -Tricosane	C ₂₃ H ₄₈	0.88
22.43	1.07	<i>n</i> -Tetracosane	C ₂₄ H ₅₀	0.91
23.37	2.87	<i>n</i> -Pentacosane	C ₂₅ H ₅₂	0.95
24.61	16.45	<i>n</i> -Hexacosane*	C ₂₆ H ₅₄	1.00
26.18	8.77	<i>n</i> -Heptacosane	C ₂₇ H ₅₆	1.06
27.18	5.94	<i>n</i> -Octacosane	C ₂₈ H ₅₈	1.10
28.96	7.76	<i>n</i> -Nonacosane	C ₂₉ H ₆₀	1.18
29.49	7.45	<i>n</i> -Triacotane	C ₃₀ H ₆₂	1.20
31.37	4.90	Campesterol	C ₂₈ H ₄₈ O	1.27
32.34	7.12	Stigmasterol	C ₂₉ H ₄₈ O	1.31
34.50	6.15	β -Sitosterol	C ₂₉ H ₅₀ O	1.40
38.32	1.90	α -Amyrin	C ₃₀ H ₅₀ O	1.56
Total hydrocarbons%				61.66%
Total sterols%				20.07%
Total identified compounds%				81.73%
Unidentified compounds%				18.27%

*RRT: relative retention time of *n*-Hexacosane = 1 with RT = 24.61 min





Identified hydrocarbons and sterols of *C. variegatum* cv. Gold Dust by GLC

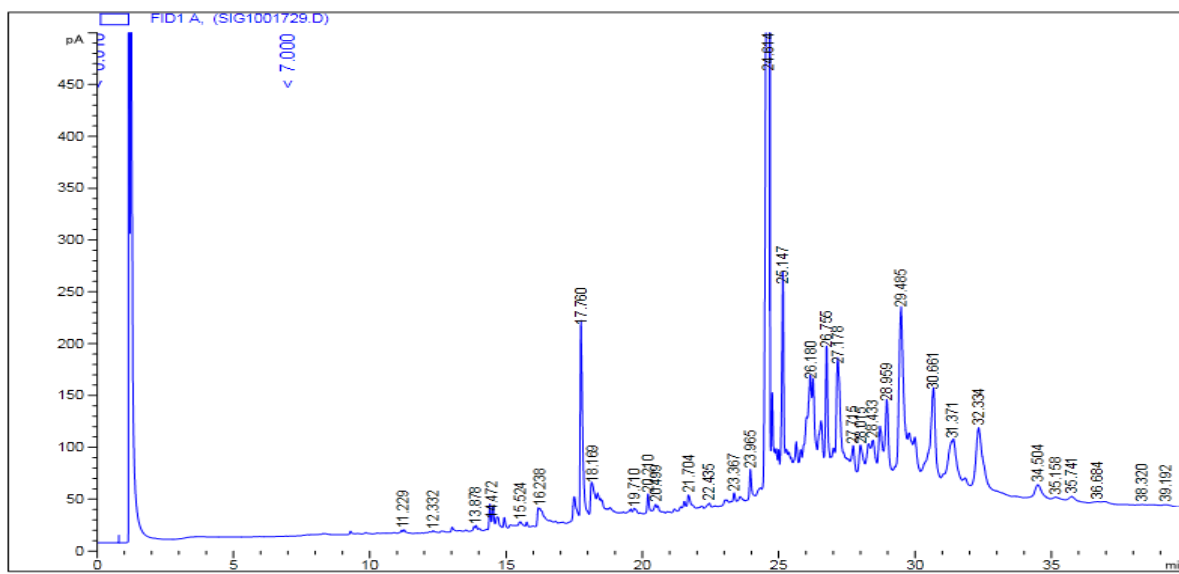


Figure 2. GLC chromatogram of USM of *Codiaeum variegatum* cv. Gold Dust

GLC analysis of the unsaponifiable matter

The unsaponifiable matter was analyzed by GLC technique together in comparison with the available authentic hydrocarbons and sterols. The analysis was recorded at the principal laboratory, NRC, Cairo, Egypt, on Pye UNIC GLC, series 304 equipped with flame ionization detector (FID). Chromatographic separation was accomplished using coiled glass column (UNICAM Pro GC, 2.8 m × 4 mm) packed with Diatomite (100 - 120 mesh) and coated with 3% Ohio Valley (OV-17). The oven temperature was programmed at 10 °C/min from 70 °C, then isothermally at 270 °C for 25 min. The nitrogen flow rate was adjusted to 30 ml/min. The detector, injector temperatures and hydrogen, air flow rates were generally adjusted to 300 °C, 280 °C and 33 ml/min, 330 ml/min, respectively

GLC analysis of the FAME

GLC analysis of FAME was carried at the principal laboratory, NRC, Cairo, Egypt adopting almost the same parameters as in unsaponifiable investigation except for the usage of a coiled glass column (UNICAM Pro GC, 1.5 m × 4 mm) packed with diatomite (100 - 120 mesh) and coated with 10% polyethylene glycol adipate (PEGA). The column oven temperature was programmed at 8 °C/min from 70 °C to 190 °C, then isothermally at 190 °C for 25 min with nitrogen flow rate at 30 ml/min.

Identification of USM and TFA

Identification of the hydrocarbons, sterols and fatty acids was carried out by comparing the relative retention times of the recorded peaks with those of the pure available authentic reference standards. Quantitative estimation was done by peak area measurement using a computing integration software (PU 4810, Philips).

Proximate Analysis

The proximate analysis of the ash, moisture, crude fiber and lipid contents was performed in the Cairo University Research Park (CURP), Faculty of Agriculture, Cairo University, Cairo, Egypt, following the standard analytical methods delineated by the AOAC (Association of Official Analytical Chemists)^{13,14}. All measurements were recorded as g/100g dry weight.

Moisture Content

About 1gm of air-dried, powder of *Codiaeum variegatum* cv. Gold Dust leaves and stems was added to a pre-weighted empty cup (W₁). The cup with the plant sample (W₂) was dried in an oven at 105 °C overnight. In the next day, the sample was cooled in a desiccator and weighed. It was then returned to the oven

for further drying. Drying, cooling, and weighing were done repeatedly at hourly interval until the sample reach a constant weight (W₃), and the moisture content was calculated from the following equation as the loss in weight that occurs when a sample is dried to a constant weight in an oven:

$$\% \text{ Moisture content} = \frac{\text{Weight of sample before drying (W}_2 - \text{W}_1) - \text{Weight of sample after drying (W}_3 - \text{W}_1)}{\text{Weight of the sample}} \times 100$$

Total Ash Value

About 1gm of air-dried, powder of *Codiaeum variegatum* cv. Gold Dust leaves and stems was added to a pre-weighted empty crucible. The crucible was introduced to a muffle furnace at 550 °C overnight. In the next day, the cup with the incinerated plant sample (turned to ash) was re-weighted and the ash value was calculated from the following equation:

$$\text{Ash value} = \frac{\text{Weight of the crucible with the ash (W}_2) - \text{Weight of the empty crucible (W}_1)}{\text{Weight of plant sample (W)}} \times 100$$

Crude Fiber Content

An empty fiber bag was heated in an oven at 100 °C for 15 min and weighted. About 1gm of air-dried, powdered leaves and stems of *Codiaeum variegatum* cv. Gold Dust was added to the pre-weighted fiber bag (Fs). Thereafter, the sample was feed in a fibertherm® apparatus (Gerhardt, Laboratory Instrument, FT12) for about 4 h, where it is digested with 1.25% H₂SO₄, followed by washing with hot water then heated with 1.25% NaOH and again washed finally heating in an oven adjusted to a temperature of 105 °C for 24 h. Thereafter the fiber bag was removed from the oven and weighted. Next, the fiber bag was transferred to pre-weighted empty cup and introduced to a muffle furnace at 550 °C for 24 hours. Finally, the whole sample was taken from the muffle and weighted (Cf). The crude fiber content was calculated as following:

$$\text{Fiber Content} = \frac{[(\text{Dfs} - \text{Fe}) - (\text{Cf} - \text{Ce})]}{\text{Weight of the sample}} \times 100$$

Fe=Weight of the empty fiber bag after heating in an oven at 100 °C

Fs= Weight of the fiber bag filled with sample

Dfs= Weight of the fiber bag filled with sample after heating in the oven at 105 °C

Cf= Weight of the whole sample after heating in the muffle at 550 °C

Ce= Weight of the empty cup

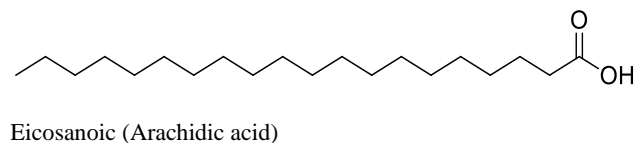
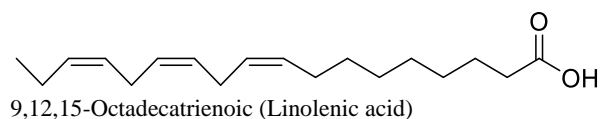
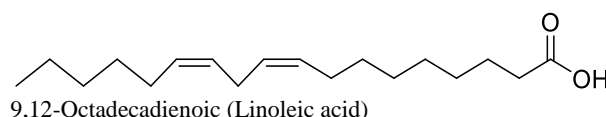
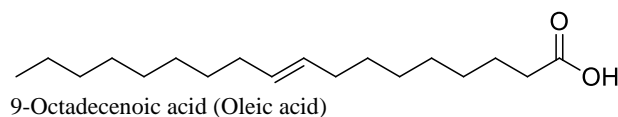
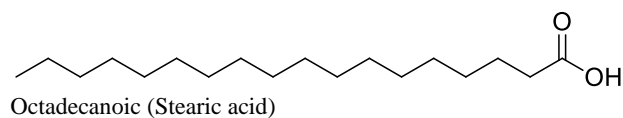
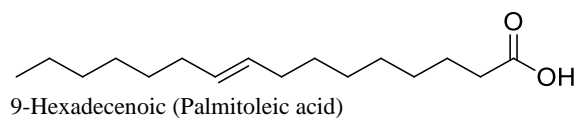
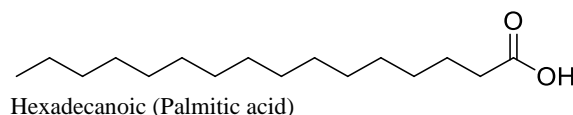
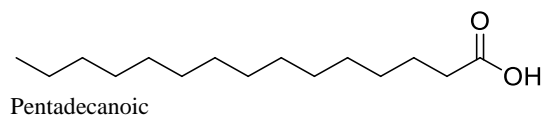
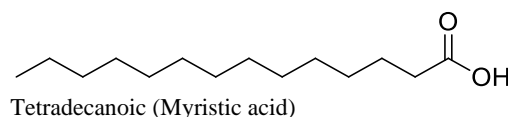
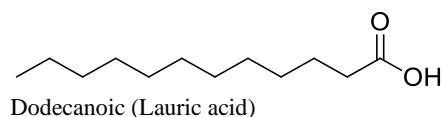
Total Fat Content

1gm of air-dried, powder of *Codiaeum variegatum* cv. Gold Dust leaves and stems were extracted with petroleum ether (2x300 ml, b.p. 60-80 °C). The extracts were filtered, pooled together, then evaporated in a pre-weighted flask under reduced pressure at 50 °C. The dried residue was cooled in

Table 3. GLC-analysis of derivatized fatty acids of *Codiaeum variegatum* cv. Gold Dust

RT	Peak area	fatty acid	Molecular formula	RRT*
12.79	13.03	Dodecanoic (Lauric acid)	C ₁₂ H ₂₄ O ₂ (12:0)	0.51
16.28	12.82	Tetradecanoic (Myristic acid)	C ₁₄ H ₂₈ O ₂ (14:0)	0.65
18.16	0.79	Pentadecanoic	C ₁₅ H ₃₀ O ₂ (15:0)	0.72
20.98	18.68	Hexadecanoic (Palmitic acid)	C ₁₆ H ₃₂ O ₂ (16:0)	0.83
21.78	6.65	9-Hexadecenoic (Palmitoleic acid)	C ₁₆ H ₃₀ O ₂ (16:1)	0.87
24.17	0.76	Octadecanoic (Stearic acid)	C ₁₈ H ₃₆ O ₂ (18:0)	0.96
25.18	27.90	9-Octadecenoic (Oleic acid)*	C ₁₈ H ₃₄ O ₂ (18:1)	1.00
25.89	10.48	9,12-Octadecadienoic (Linoleic acid)	C ₁₈ H ₃₂ O ₂ (18:2)	1.03
26.87	4.95	9,12,15-Octadecatrienoic (Linolenic)	C ₁₈ H ₃₀ O ₂ (18:3)	1.07
30.04	0.82	Eicosanoic (Arachidic acid)	C ₂₀ H ₄₀ O ₂ (20:0)	1.19
Saturated fatty acid				46.90%
Unsaturated fatty acid				49.98%
Un identified compounds				3.12%

*RRT: relative retention time of 9-Octadecenoic (Oleic acid) = 1, RT =25.18 min



Identified fatty acids of *C. variegatum* cv. Gold Dust by GLC

<http://aprh.journals.ekb.eg/>

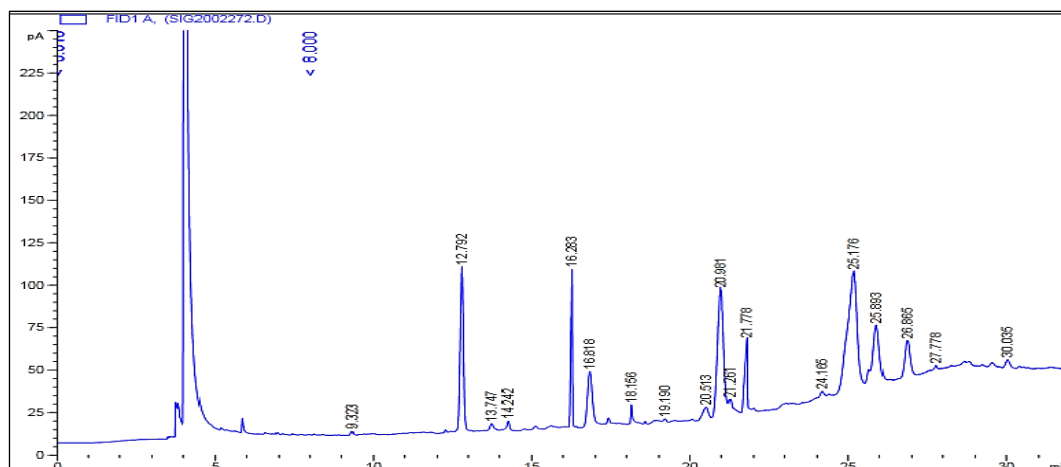


Figure 3. GLC chromatogram of derivatized fatty acids of *Codiaeum variegatum* cv. Gold Dust

a desiccator, weighted, and the total fat content was calculated as following:

% Fat Content

$$= \frac{\text{Weight of the flask with the fat (W2)} - \text{Weight of the empty flask (W1)}}{\text{Weight of the sample}} \times 100$$

RESULTS

Qualitative phytochemical screening

Qualitative identification of the phytoconstituents present in various fractions of *Codiaeum variegatum* cv. Gold Dust leaves and stems were performed as per standard procedures stated before. The results revealed the presence of flavonoids in all tested fractions and the residue, while anthraquinones and coumarins were absent in all tested samples. Alkaloid and/or nitrogenous bases, carbohydrates, saponins, and tannins were present in the residue but absent in DMC, EtOAc and BuOH fractions. Cardiac glycosides were present in the EtOAc and the residue. Terpenes and/or sterols were present in DCM only. The collective results of the qualitative phytochemical screening are summarized in **Table 1**.

Quantitative investigation of the lipoidal matter

Fractionation of the petroleum ether extract of *Codiaeum variegatum* cv. Gold Dust leaves and stems yielded 50% unsaponifiable matter (hydrocarbons and sterols) and 45% fatty acids (saponifiable matter). The results of GLC analysis of unsaponifiable matter are presented in **Table 2** and **Figure 2**, which tentatively revealed the identification of sixteen aliphatic, saturated hydrocarbons (61.66%). It was observed that *n*-hexacosane ($C_{26}H_{54}$) was the major identified hydrocarbon (16.45%). Concerning the sterol

composition, three sterols (campesterol, stigmasterol, and β -sitosterol) were identified comprising about 20.07%, while just one triterpene (α -amyrin) was detected in 1.90%. On the other hand, the results of fatty acid methyl esters analysis (FAME) are presented in **Table 3** and **Figure 3**, which tentatively revealed the identification of four unsaturated fatty acids (49.98%) and six saturated ones (46.90%). It was observed that 9-octadecenoic (oleic acid) represents the major identified unsaturated fatty acid (27.90%), while hexadecanoic (palmitic acid) represents the major saturated fatty acid (18.68%).

Proximate analysis

The proximate composition of *Codiaeum variegatum* cv. Gold Dust leaves and stems was carried out in accordance with the Association of Official Analytical Chemists (AOAC, 1986, 2000) standard procedures. The assayed parameters include; crude fiber, total ash, fat, and moisture content. The results (**Figure 4**) revealed considerable level of crude fibers (21.7%), moderate ash (14.9%) and moisture values (10.77%), in addition to about 1.0 g/100g dry powder of fat content.

DISCUSSION

Codiaeum variegatum cv. Gold Dust, leaves and stems, various fractions; DCM, EtOAc, BuOH, and the residue, were assessed for the presence of various metabolites following the standard methods mentioned before. The results showed the presence of various phytoconstituents as carbohydrates, flavonoids, tannins, alkaloids, cardiac glycosides, terpenes and/ or sterols which are almost deemed for significant biological activities as antioxidant, anti-inflammatory, antimicrobial, and anticancer¹⁶⁻¹⁹. Hence, the plant under investigation may be correlated to a great extent

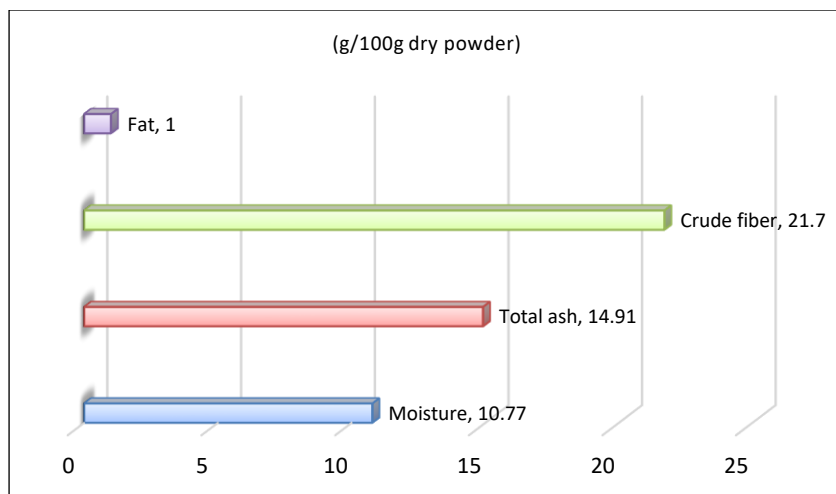


Figure 4. Proximate analysis of *Codiaeum variegatum* cv. Gold Dust leaves and stems

with a panel of unexplored biological activities. In all, encourage to investigate an in-depth study to isolate and identify the correlated phytoconstituents.

From the prior qualitative screening results, the non-polar soluble fraction showed a considerable prevalence of sterols and/ or triterpenes, hence the petroleum ether extract of *C. variegatum* cv. Gold Dust was subjected to quantitative estimation of its lipoidal matter content using GC technique. The qualitative identification of the hydrocarbons, sterols, and fatty acids was accomplished by comparing the relative retention times of the recorded peaks with those of the pure available authentic reference standards, while the quantitative estimation was conducted by relative peak area integration. The results showed the abundance of the saturated hydrocarbon, *n*-hexacosane which has worthy noted for its antimicrobial activity²⁴ on the other hand, oleic acid was identified as the prime, unsaturated fatty acid, which is well known for its contribution in reducing the risk of high blood cholesterol levels and coronary heart disease (CHD) as the confirmed data reported by Preedy and Waston.²⁵ In addition, Harada, reports the antioxidant and antitumor activities for Palmitic acid²⁶, the most abundant saturated fatty acid. Conclusively, the results spot the light on the valuable, expected biological importance related to the identified lipoidal components.

Moreover, the proximate analysis revealed considerable level of crude fibers (21.7 g/100g dry powder). A result which may be correlated, at least in part, to improving digestion, high water-absorption capacity, acquiring bulk stool and prevention of constipation²⁷. Consequently, *C. variegatum* cv. Gold Dust leaves and stems may be useful in controlling digestive disorders, lower cholesterol level, and protect against cancer²⁸. On the other hand, the moderate ash

value showed up that the plant has appreciable content of mineral elements, while measuring the ash content is a relevant parameter for the measurement of mineral matter that may has a crucial contribution in various pharmacological effects²⁹.

CONCLUSION

The qualitative phytochemical screening revealed that *Codiaeum variegatum* cv. Gold Dust leaves and stems are valuable source of a variety of bioactive secondary metabolites, crude fibers, mineral elements, unsaturated fatty acids and sterols. In line with the global demands for discovery of bioactive metabolites from natural source, therefore the plant under investigation could be incorporated as potential sources of supplements in dietary formulations. Nevertheless, extensive toxicity study needs to be done to ascertain the safety levels. In all, it is worthy notice that is the first report for phytochemical screening, lipoidal investigation and proximate analysis of *Codiaeum variegatum* cv. Gold Dust leaves and stems cultivated in Egypt.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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