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Original Research Article

Phytochemical screening of *Catha edulis* (Khat) and its effect on the development of chick embryo

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Abstract

Purpose: *To identify the chemical constituents of Catha edulis (khat) and evaluate its embryotoxicity in chicks.*
Methods: *The methanol extract of Catha edulis was analyzed by gas chromatography–mass spectrometry (GC–MS). One hundred fertile chicken eggs of Gallus gallus were divided into ten groups and injected in the air chamber with different concentrations of methanolic extract of Catha edulis (control, 1.21, 2.43, 3.63, 4.86, 6.08, 7.30, 8.51, 9.73, and 10.95 mg/mL). On the 7th day of incubation, the eggs were opened to determine the median lethal dose (LD_{50}) and embryotoxicity.*
Results: *An analysis of the methanolic extract from khat leaves revealed the presence of thirty-one chemical constituents, collectively comprising 76.66 % of the extract. These constituents include alkaloids, terpenoids, fatty acids and their derivatives, fatty alcohols and their derivatives, glycosides, and vitamins. Notably, cathine and cathinone emerge as the key contributors to the pharmacological and toxic effects linked to khat consumption. The median lethal dose (LD_{50}) for chicken embryos was 5.573 mg/mL. The results showed a teratogenic effect at concentrations of 4.86, 6.08 and 7.30 mg/mL. Embryo deformation and hematomas were noted at concentrations of 8.51, 9.73 and 10.95 mg/mL.*
Conclusion: *Catha edulis is toxic to chicken embryos and has a negative impact on fetal organization that might be due to cathine and cathinone.*

Keywords: *Catha edulis, Chick abnormalities, Chick embryo, Khat, Phytochemical screening*

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INTRODUCTION

Catha edulis (khat) is a large, flowering shrub (1–5 m) that grows in arid environments, belongs to the family Celastraceae, and is cultivated in East Africa and the Arabian Peninsula [1]. Khat is categorized by the World Health Organization as a drug of abuse due to its association with a range of health problems [2]. The documented methods of khat consumption are diverse and include not only chewing fresh leaves but also chewing dried leaves or drinking khat as a tea-like beverage [3]. Khat extraction causes elevation of estradiol levels and markedly sexual motivation in the female rats that suggesting it could serves as a potential to enhance the female sexual desire [4]. There is growing biological evidence to suggest that chronic khat leaf use is associated with memory impairments and reduced cognitive flexibility. Its perceived

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effects encompass augmenting mental capacity, enhancing physical strength, acting as a facilitator of social entertainment, promoting a sense of cheerfulness, anecdotally, and being associated with heightened sexual experiences [3]. Cathinone disrupts the pituitary cells causing alterations in the gonadotrophs, lactotrops, and corticortrophs of the experimental animals [5]. Although only a limited number of reports directly associate deaths among khat chewers with khat-induced heart failure, a substantial body of evidence underscores the deleterious cardiovascular consequences of khat use [6]. Numerous reports strongly advise against the use of khat leaves during pregnancy due to their potential adverse effects, encompassing teratogenic consequences and a reduction of food intake [7]. Similar to amphetamine, cathinone exerts adverse myocardial effects by altering heart rate, blood pressure, and vascular function [6]. Khat leaves have been associated with anorexia and diminished food absorption in the gastrointestinal tract, emphasizing their potential risks during pregnancy [2]. Despite ongoing investigations into the impact of khat leaves on embryos, the existing literature on their effects on embryos and fetuses is limited. Accordingly, the present study aims to assess the embryotoxic effects of khat leaves using a chick embryo model.

EXPERIMENTAL

Preparation of extract

Khat leaf powder (5 g) underwent an exhaustive extraction process using 150 mL of methanol in a Soxhlet apparatus, with extraction lasting 9 to 10 hours. The resulting extract was concentrated by evaporation under reduced pressure, yielding brown viscous masses (0.608 g; 12.17 %). The samples were then stored in the dark at 4 °C for subsequent analyses, including gas chromatography–mass spectrometry (GC–MS) analysis and assessment of their potential effects in the developing chick embryo.

GC–MS characterization of the extracts

The GC–MS analysis of the methanolic extract was performed using a Shimadzu GC–MS QP2010 system equipped with an AOC-20i+s liquid injector and computer-controlled software. Separation was achieved on a ZB-5MSi capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). Helium was used as the carrier gas at a flow rate of 1.7 mL/min. The oven temperature was initially set at 50 °C, then increased at a rate of 15 °C/min to 290 °C and maintained at this temperature for 30 min. Mass spectrometric detection was carried out using a Shimadzu QP2010 Ultra detector operating at an ionization energy of 70 eV.

Identification of phytochemical constituents

The constituents were primarily identified by GC–MS through comparison of their retention indices with those of authentic standards available in the laboratory or with reference values reported in the literature. Further confirmation of compound identities was achieved by comparing the mass spectral fragmentation patterns with those stored in the NIST 17 and Wiley 229 spectral libraries.

Design and processing

The experiments were designed to study chick embryotoxicity. Experiments were carried out on the 7th day of incubation. The methanolic extract of khat (*Catha edulis*) was dissolved in dimethyl sulfoxide (DMSO; 0.05 %) for injection. Fertile chicken eggs of *Gallus gallus domesticus* were obtained from local AlUla farms, Madinah, Saudi Arabia. The toxicity test of extracted *Catha edulis* on the developing chick embryo was done according to Henry and Wyatt [8]. One hundred fertile chicken eggs were divided into ten treatment groups (ten eggs in each group). The mean weight of eggs was 40 ± 1.25 g. The first group was the control (G1), which was injected with DMSO (0.05 %). The treatment groups were exposed to different concentrations of extract/egg as follows: G2 (1.217 mg/mL), G3 (2.434 mg/mL), G4 (3.634 mg/mL), G5 (4.868 mg/mL), G6 (6.085 mg/mL), G7 (7.302 mg/mL), G8 (8.519 mg/mL), G9 (9.736 mg/mL), and G10 (10.953 mg/mL). The single air sac injection of extracted *Catha edulis* was done by drilling a hole for a single dose only before the onset of incubation. All fertile eggs in different treatments were injected with a 10 µL volume in the air chamber. The injection site was sealed with a drop of paraffin wax and labeled with a pencil. After 7 days of incubation, the chick embryos were photographed and examined for developmental abnormalities with the aid of a dissecting microscope.

Data analysis

Mortality data were transformed to probit and subjected to linear regression analysis to determine the median lethal dose (LD_{50}) of *Catha edulis* extract [8].

RESULTS

The analysis of khat leaves involved the separation and identification of various

phytoconstituents through GC–MS. Figure 1 depicts the proportional chromatograms of khat extracts as outlined in Table 1 that reveals the presence of various compounds, notably cathine and cathinone. The cumulative area percentage for the identified components reached 76.66 %.

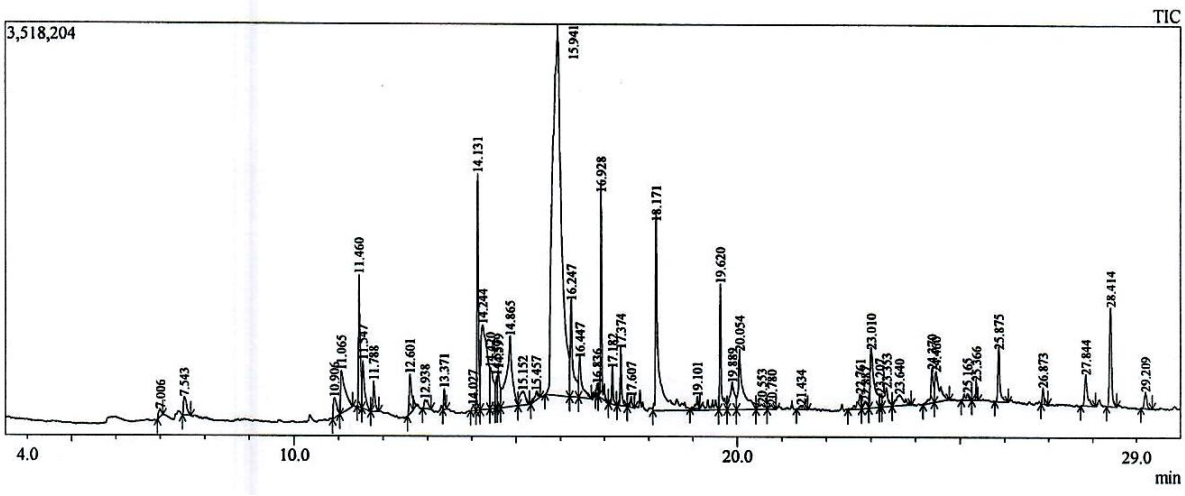
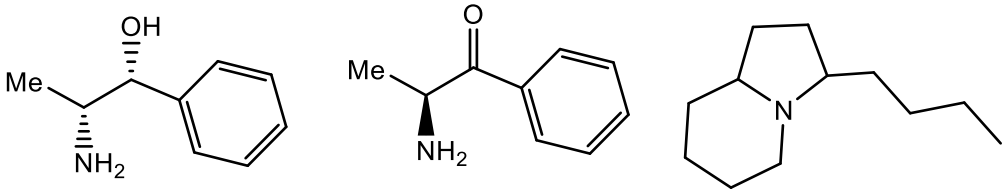


Figure 1: Representative typical GC–MS total ion current (TIC) chromatograms of the methanolic extract of khat leaves

Table 1: Chemical composition of the methanolic extract of khat leaves

Name of compound	No	Retention time	Area (%)	Mol. formula	M. wt	Figure
Cathine	1	11.07	2.01	$C_9H_{13}NO$	151	2
Cathinone	2	10.91	0.74	$C_9H_{11}NO$	149	2
3-Butylindolizidine	3	17.60	0.21	$C_{12}H_{23}N$	181	2
4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one	4	15.46	0.27	$C_{13}H_{20}O_3$	224	3
Citronellol	5	19.62	2.06	$C_{10}H_{20}O$	156	3
Phytol, acetate	6	16.93	2.69	$C_{22}H_{42}O_2$	338	3
Trans-Phytol	7	17.18	0.54	$C_{20}H_{40}O$	296	3
Squalene	8	25.37	0.29	$C_{30}H_{50}$	410	3
n-Hexadecanoic acid	9	18.17	6.82	$C_{16}H_{32}O2$	256	4
Octadecanoic acid	10	20.05	3.00	$C_{18}H_{36}O_2$	284	4
(*E*)-13-Docosenoic acid	11	21.43	0.18	$C_{22}H_{42}O_2$	338	4
9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	12	22.76	0.56	$C_{18}H_{30}O_2$	278	4
d,l-Xylitol, 1-O-dodecanoyl	13	14.03	0.20	$C_{17}H_{34}O_6$	334	5
1,3-Dihydroxypropan-2-yl palmitate	14	23.01	2.57	$C_{19}H_{38}O_4$	330	5
Ethyl linolenoate	15	24.46	1.54	$C_{20}H_{34}O_2$	306	5
Z,Z,Z-7,10,13-Hexadecatrienal	16	19.89	1.35	$C_{16}H_{26}O$	234	6
(*S*, *S*, *S*, *S*)-1,1'-Bicyclopentyl-2,2'-dicarboxaldehyde	17	20.55	0.39	$C_{12}H_{18}O_2$	194	6
1-Heptacosanol	18	25.88	1.26	$C_{27}H_{56}O$	396	7
n-Tetracosanol	19	27.84	0.96	$C_{24}H_{50}O$	354	7
4-Methylpentyl pentanoate	20	11.79	0.65	$C_{11}H_{22}O_2$	186	7
4-Methylpentyl hexanoate	21	13.37	0.29	$C_{12}H_{24}O_2$	200	7
Octacosyl acetate	22	26.88	0.27	$C_{30}H_{60}O_2$	452	7
Melissyl acetate	23	29.21	0.45	$C_{32}H_{64}O_2$	480	7
.*β*.-D-Glucopyranoside, methyl	24	14.24	5.84	$C_7H_{14}O_6$	194	8
D-Mannose	25	14.42	1.56	$C_6H_{12}O_6$	180	8
4-Methylmannitol	26	15.94	34.84	$C_7H_{16}O_6$	196	8
2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	27	7.54	0.59	$C_6H_8O_3$	128	9
Vitamin E	28	28.41	2.33	$C_{29}H_{50}O_2$	430	9
1,4-Eicosadiene	29	17.37	0.89	$C_{20}H_{38}$	278	9
Z,Z,Z-4,6,9-Nonadecatriene	30	25.17	0.29	$C_{19}H_{34}$	262	9
2-methylhexacosane	31	23.37	1.02	$C_{27}H_{56}$	380	9
Total			76.66			



cathine

1

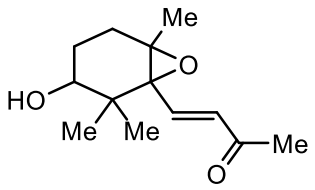
Cathinone

2

3-Butylindolizidine

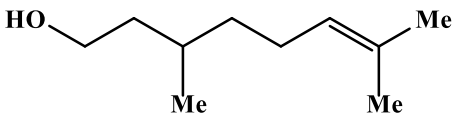
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Figure 2: The alkaloid compounds of the methanolic extract of khat leaves.



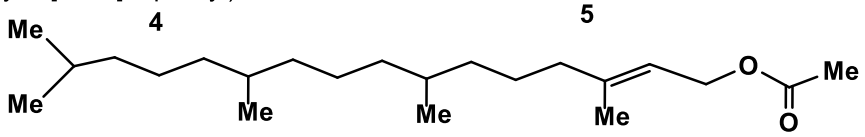
4-(3-Hydroxy-2,2,6-trimethyl-7-oxa-bicyclo[4.1.0]hept-1-yl)-but-3-en-2-one

4



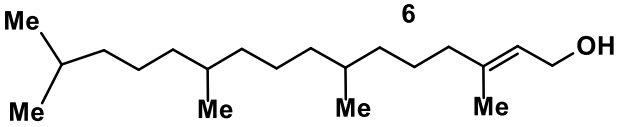
citronellol

5



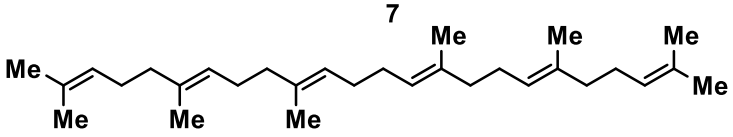
Phytol, acetate

6



Trans-Phytol

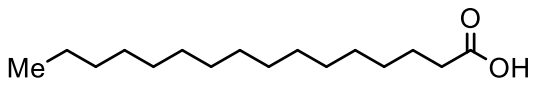
7



Squalene

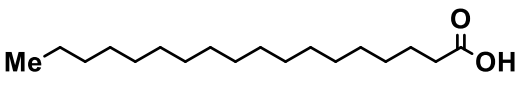
8

Figure 3: The terpenoid compounds of the methanolic extract of khat leaves



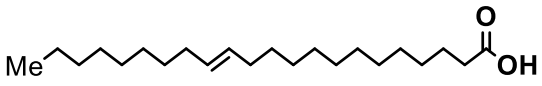
n-Hexadecanoic acid

9



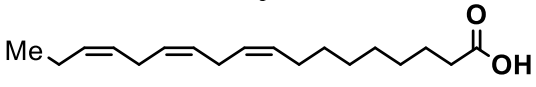
n-Octadecanoic acid

10



(*E*)-13-Docosenoic acid

11



(Z,Z,Z)-9,12,15-Octadecatrienoic acid

12

Figure 4: The fatty acid compounds of the methanolic extract of khat leaves

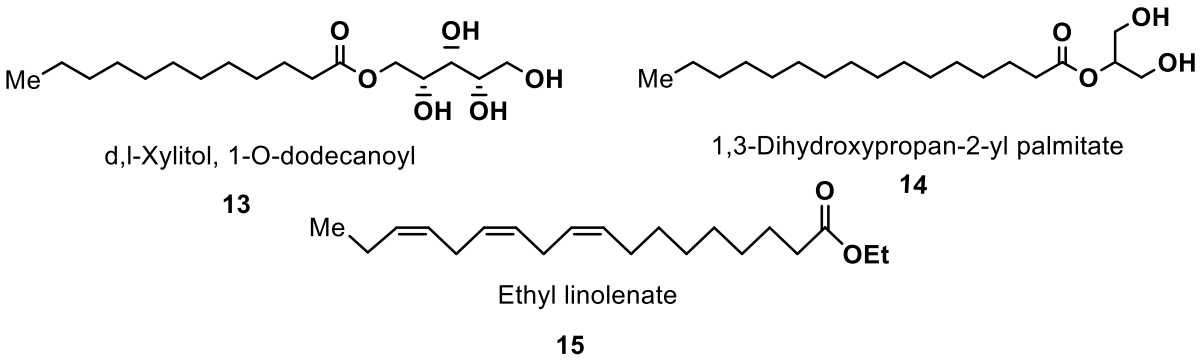


Figure 5: The ester of fatty acid compounds of the methanolic extract of khat leaves

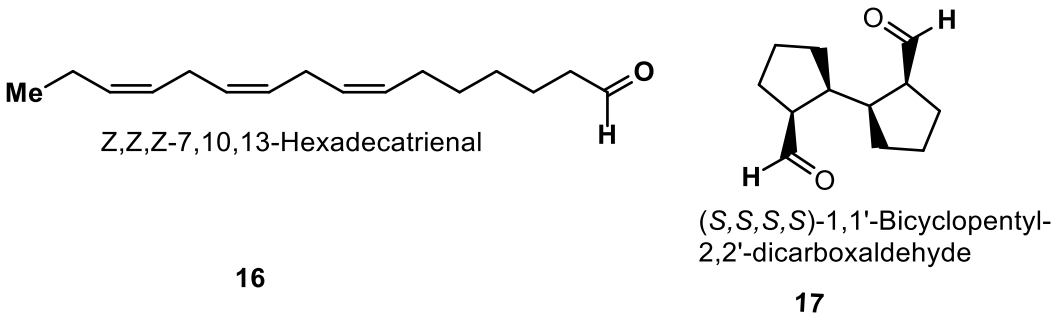


Figure 6: The long-chain aldehyde compounds of the methanolic extract of khat leaves

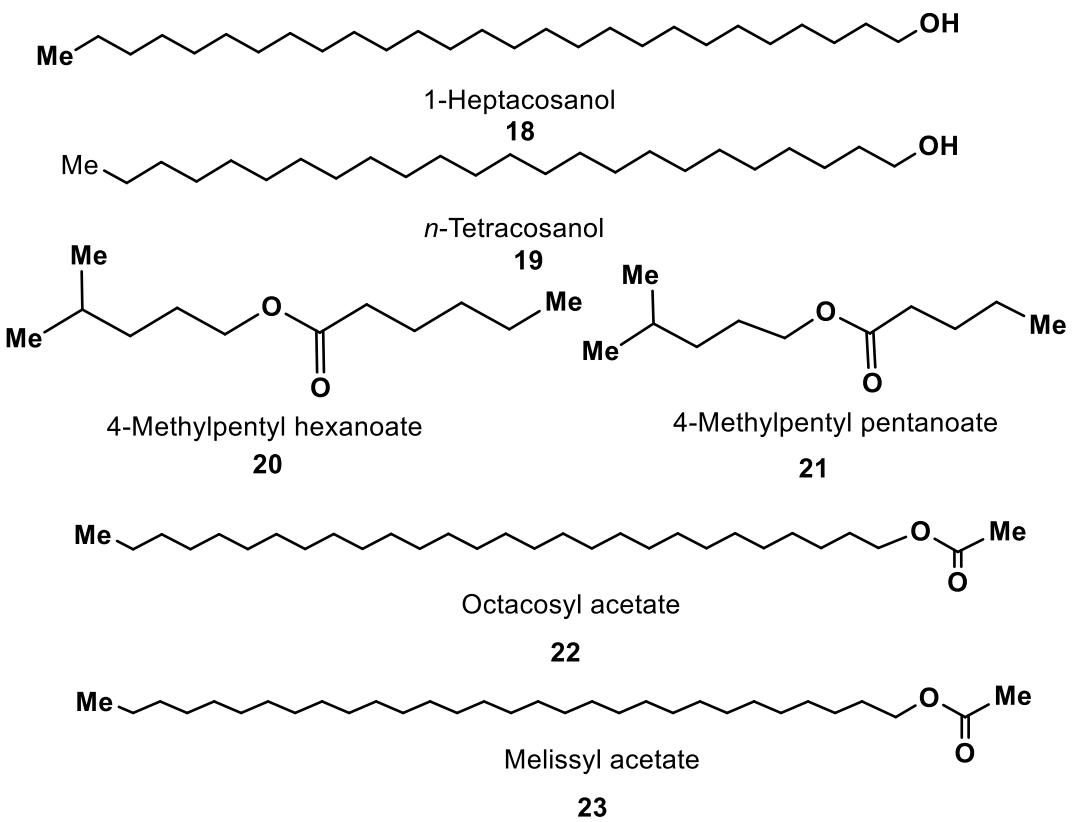


Figure 7: The long-chain alcohols and their ester compounds of the methanolic extract of khat leaves

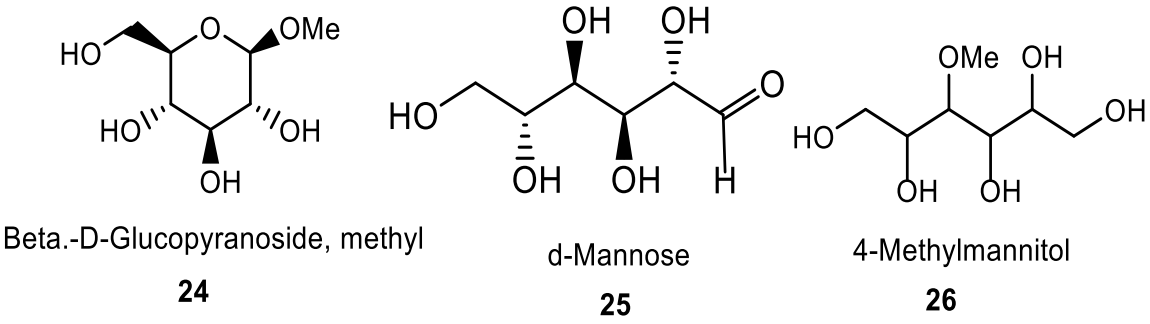


Figure 8: The sugar compounds of the methanolic extract of khat leaves

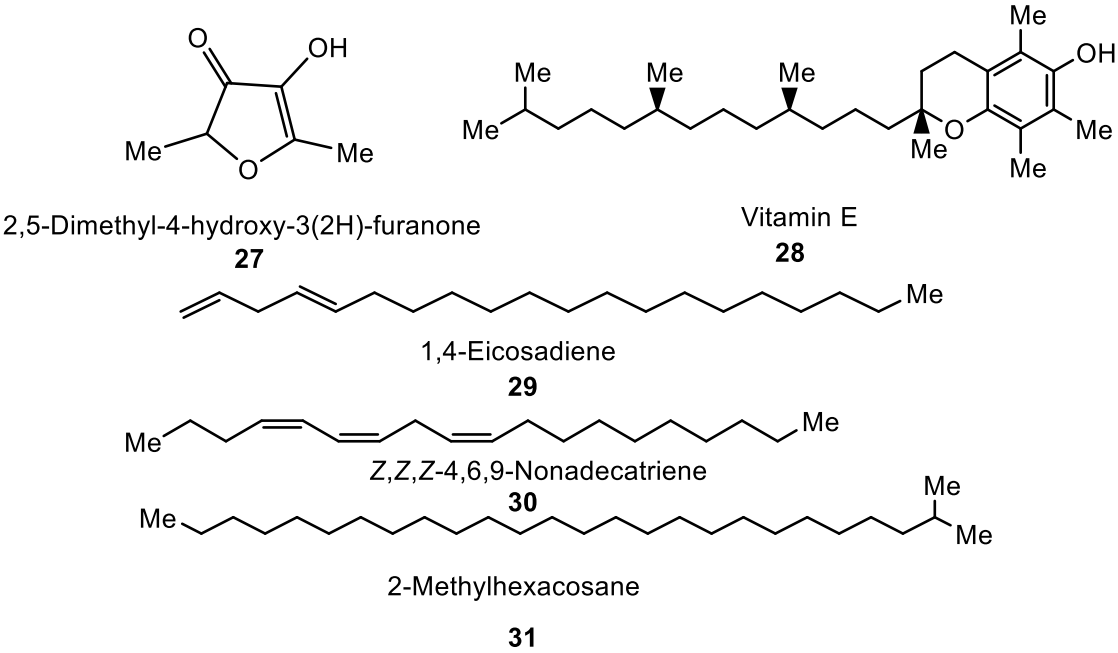


Figure 9: The furan, pyran and hydrocarbon compounds of the methanolic extract of khat leaves

Toxicological and morphological observations

The median lethal dose (LD_{50}) for chicken embryos was determined to be 5.573 mg/mL after the 7th day of incubation and exposure to a single dose of *Catha edulis* extract (Figure 10). The morphological observation of the developing control embryos on the 7th day of incubation revealed a well-developed extra-embryonic vascular plexus. It contains right and left vitelline arteries as well as the anterior and posterior vitelline veins. Well-developed blood vessels throughout the vascular area surrounding the embryo that terminates peripherally, delineating the area vasculosa from the non-vascular vitelline area (Figure 11 A). The chick embryos at low concentrations of *Catha edulis* extract in G2 (1.217 mg/mL), G3 (2.434 mg/mL), and G4 (3.634 mg/mL) showed normal morphological appearance without gross developmental abnormalities at the level of camera photography (Figure 11 B–D). The chick embryo in the treated group G5 (4.868 mg/mL) suffered from edema in the telencephalon region (Figure 11 E). Developmental delay and teratogenic effects of *Catha edulis* extract were observed in the chick embryos in group G6 (6.085 mg/mL) and the paleness of the embryonic body in G7 (7.302 mg/mL; Figure 12A, B). In *Catha edulis* extract-exposed embryos at high concentrations revealed rudimentary blood vessels, paleness, and embryo deformation. Also, hematomas, peripheral accumulation of blood, and disorganization of the blood vessels were noted in the area vasculosa in G8 (8.519 mg/mL), G9 (9.736 mg/mL), and G10 (10.953 mg/mL; Figure 12 C, D, E). Microscopic examination of treated embryos in G5 (4.868 mg/mL) or G6 (6.085 mg/mL) showed deformation in the brain and the eye was not formed (Figure 13 B, C, D) as compared to the control group (Figure 13 A).

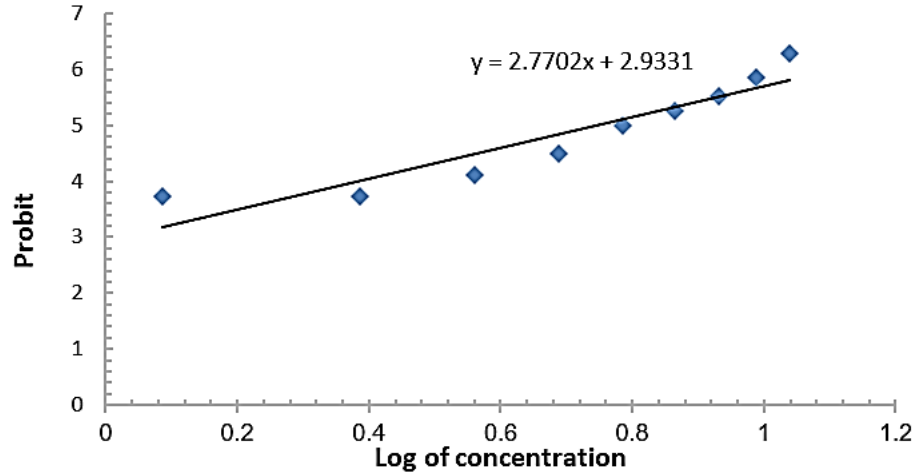


Figure 10: Determination of median lethal dose (LD_{50} = 5.573 mg/mL) on the 7th day of incubation after exposure to *Catha edulis* (khat) extract

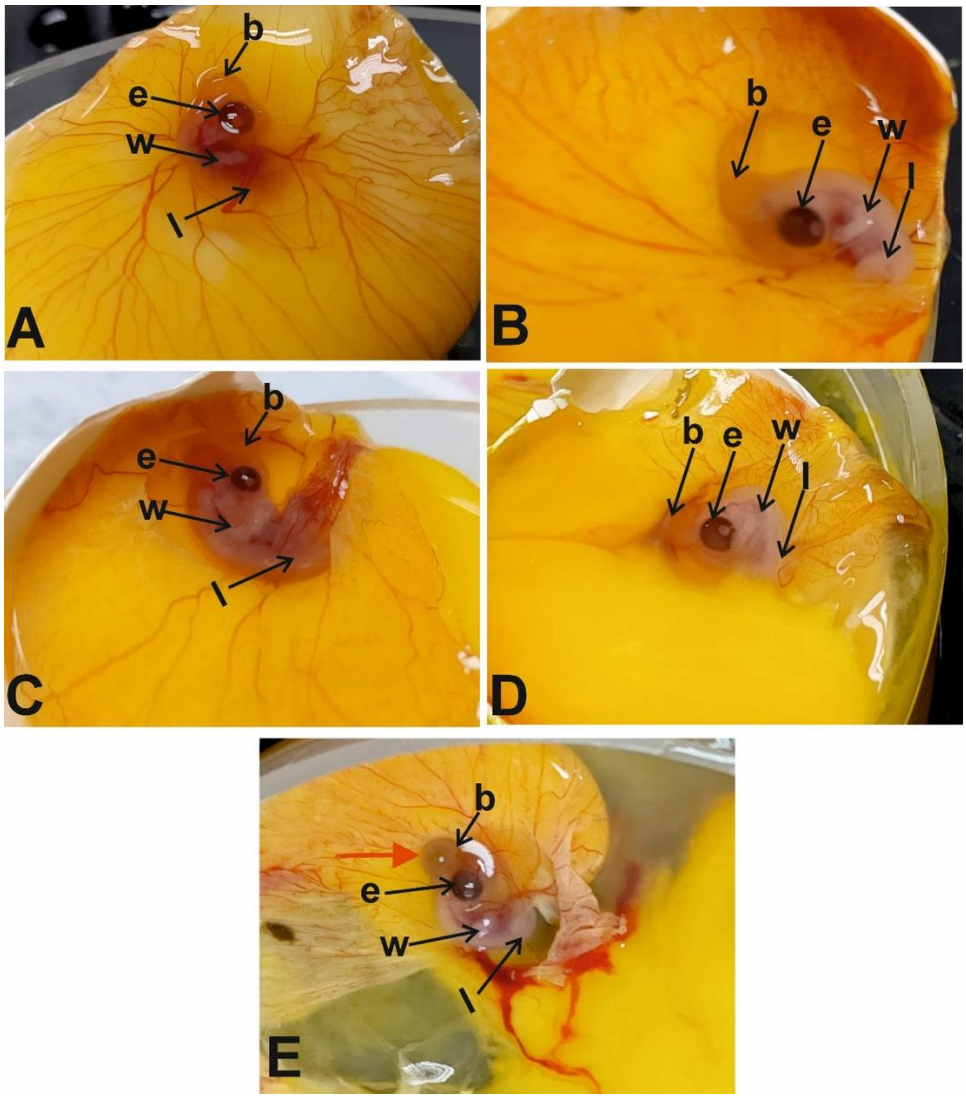


Figure 11: Photographs show the effect of *Catha edulis* extract on the developing chick embryo on the 7th day of incubation. The morphology of chick embryos appears normal in G1 (A), G2 (B), G3 (C), and G4 (D). G5 (E) showed edema in the telencephalon region (red arrow). Brain (b), eye (e), wing (w), and leg (l)

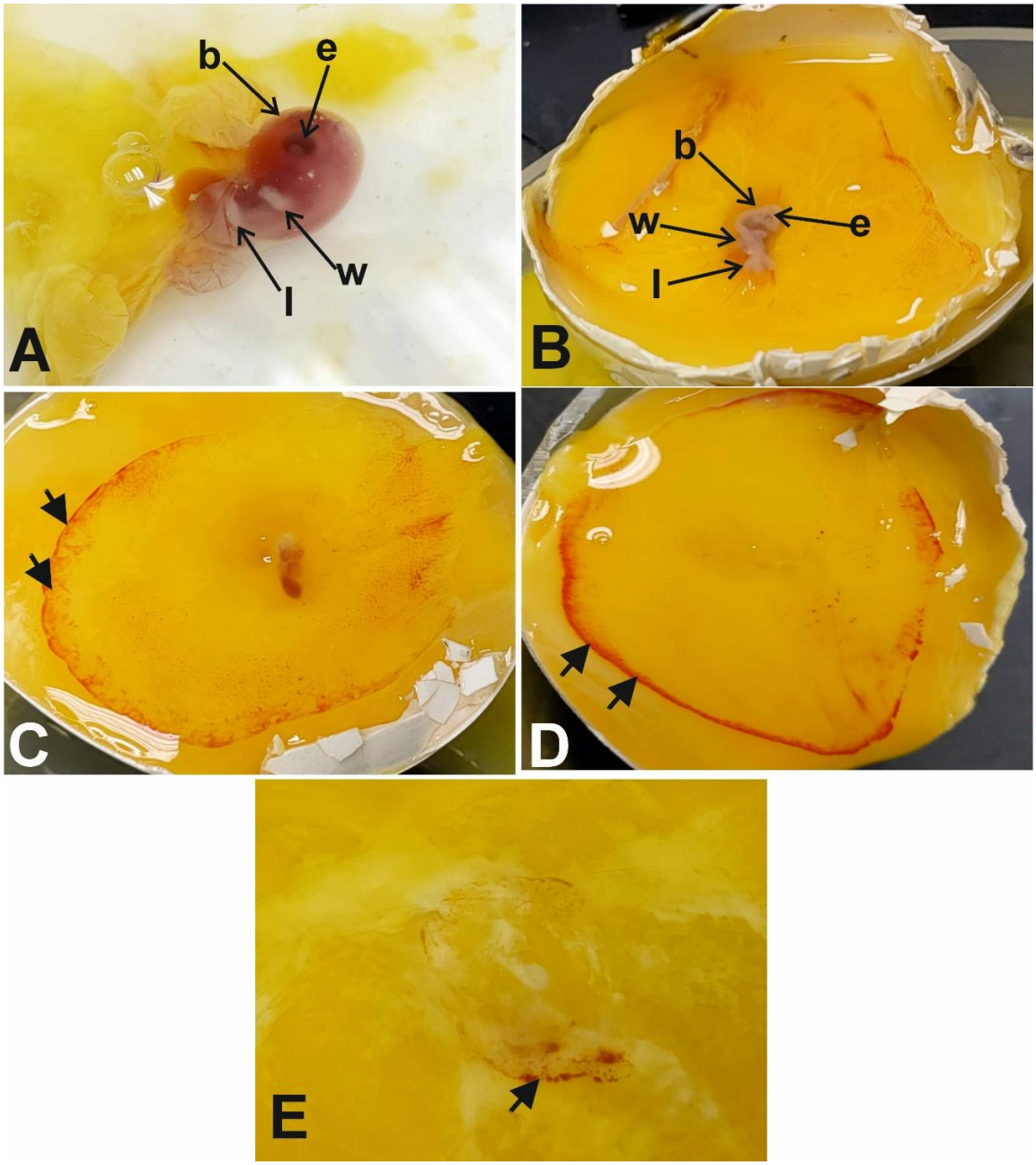


Figure 12: Photographs show the effect of *Catha edulis* methanol extract on the developing chick embryo on the 7th day of incubation. Developmental delay was noted in G6 (A) and G7 (the paleness of the embryonic body) (B). Resorbed chick embryos were detected with the peripheral accumulation of blood (arrows) in G8 (C), G9 (D), and G10 (E). Brain (b), eye (e), wing (w), and leg (l)

DISCUSSION

Phytochemical investigations of khat leaves have resulted in the isolation and identification of a diverse range of secondary metabolites, including alkaloids (1–3), terpenoids (4–8), fatty acids (9–12), fatty acid esters (13–15), long-chain aldehydes (16–17), long-chain alcohols and their esters (18–23), sugars (24–26), and various furan, pyran, and hydrocarbon derivatives (27–31). A single air sac injection of *Catha edulis* methanol extract caused toxicity in chick embryos after 7 days of incubation, except in groups G1–G4. The chick embryo assay is an established model for evaluating embryotoxicity, teratogenicity, growth retardation, developmental abnormalities, and mortality [8]. These findings support evidence that khat exposure during pregnancy may reduce uteroplacental blood flow, impair fetal growth, lower birth weight, and cause teratogenic effects [9]. Reduced maternal food intake may further worsen fetal growth restriction [7].

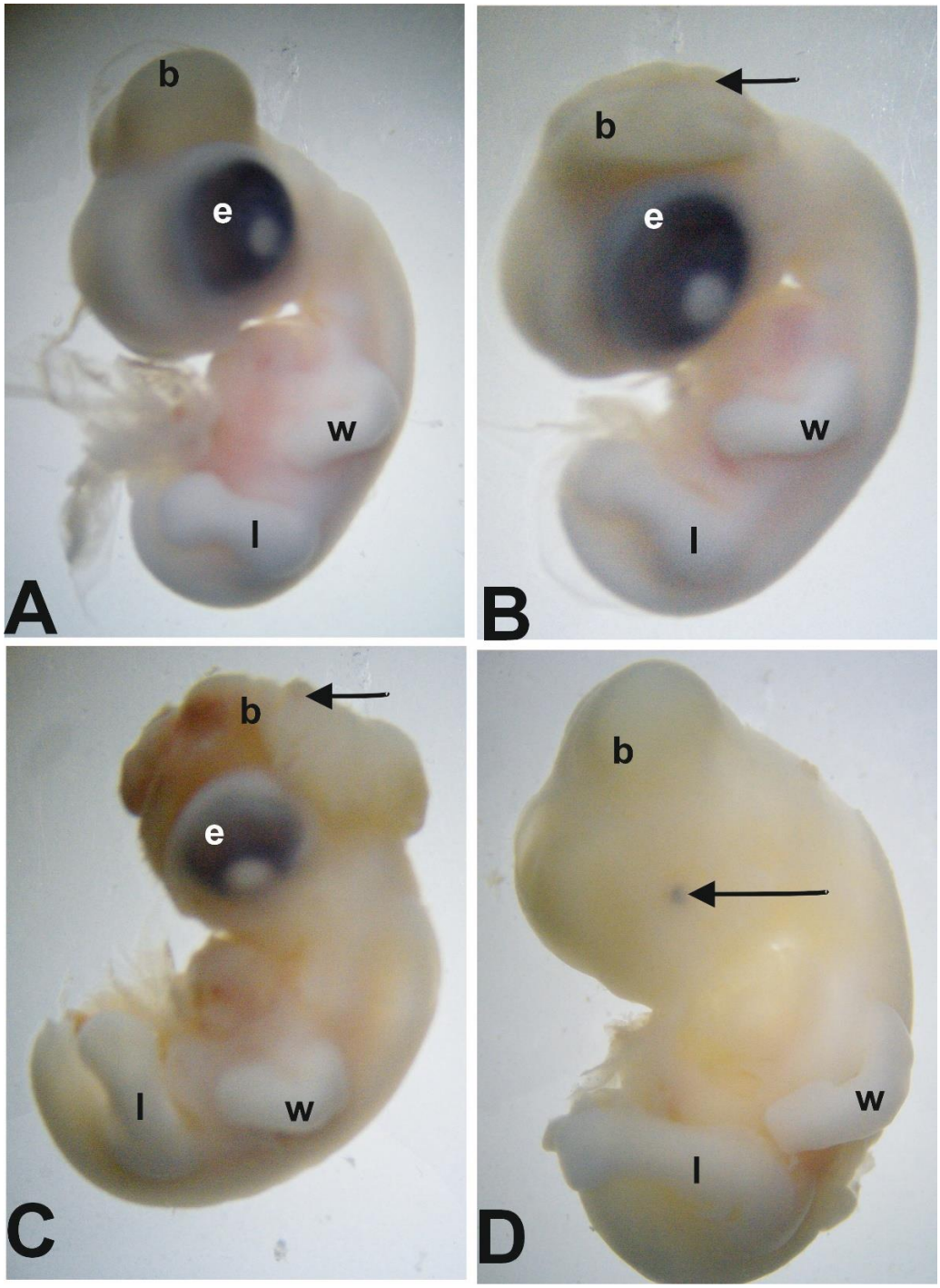


Figure 13: Photographs show the effect of *Catha edulis* (khat) methanol extract on the developing chick embryo on the 7th day of incubation. Deformation in the brain (arrows in B, C) and the eye was not formed (arrow in D). Brain (b), eye (e), wing (w), and leg (l)

Administration of *Catha edulis* causes chick embryotoxicity and fetotoxicity as observed in previous studies. Pregnant rats exposed to khat exhibited a decrease in the percentage of pregnancy rates, disorganization of the vascular tree, growth retardation, a decrease in the number of implantation sites and fetuses, post-implantation losses, low birth weight, and crown-rump length [9, 10].

Also, significant risk for preterm labor, labor induction, a significantly lower mean hemoglobin concentration, a risk for preeclampsia, a significant risk of delivering fetuses with low birth weight, risks for perinatal mortality, retardation of growth rate, and congenital malformations [11]. Teratogenic chick embryos were observed under the treatment of *Catha edulis*. Regular consumption of khat by pregnant women causes genotoxic effects for the fetus and

musculoskeletal abnormalities [12], where it still attributes retarded fetal growth in a dose-dependent manner, and an increased number of rat embryos that died while still in the uterus [13]. The observed toxicity may result from *C. edulis* bioactive constituents, particularly sympathomimetic alkaloids associated with vasoconstriction and adverse development [3,14].

Khat leaves contain cathinone ((S)-(−)-α-aminopropiophenone), which has been recognized as a psychoactive ingredient and resembles amphetamine in its effects on the central and peripheral nervous system and behavior [14]. The effect of *Catha edulis* extract might result from the nature of cathinone, which stimulates the release of catecholamines at synaptic sites and stimulates D2 receptors, then inhibits NMDA-mediated glutamate transmission [14,15]. Also, khat effects may be due to the organophosphates (use of pesticides during cultivation), which may be related to the effects of khat components cathinone and cathine [16]. Deformation in the chick brain embryo was documented, where khat chewing effects on the central nervous system include neurocognitive impairments, abnormal social behavior, euphoria, insomnia, excitability, locomotor hyperactivity, anxiety, and psychological dependency [17]. Thus, the observed embryonic brain abnormalities may result from the combined effects of khat's psychoactive constituents and, potentially, contaminating agricultural chemicals.

CONCLUSION

The methanolic extract of *Catha edulis* leaves has revealed thirty-one chemical constituents, collectively constituting 76.66 % of the extract. These constituents encompass alkaloids, terpenoids, fatty acids and their derivatives, fatty alcohols and their derivatives, glycosides, and vitamins. Significantly, cathine and cathinone stand out as the primary contributors to the pharmacologic and toxic effects associated with *Catha edulis* consumption. The act of khat-chewing, which introduces these compounds into the system, has been correlated with a spectrum of health issues. The methanolic extract of *Catha edulis* has shown significant toxicity to chicken embryos and has a negative impact on fetal organization.

DECLARATIONS

Acknowledgement/Funding

The authors extend their sincere thanks to Mr. Majed M. Al-Balawi, Department of Forensic Chemistry, General Administration of Criminal Evidences, Public Security, KSA, for the leaf extract and GC–MS analysis.

Ethical approval

The protocol was approved by the Ethics Committee of the Faculty of Science, Sohag University, Egypt (No. CSRE-3-25).

Availability of data and materials

The corresponding author can provide the datasets used and/or analyzed in this study upon reasonable request.

Conflict of interest

No conflicts of interest are associated with this study.

Contribution of authors

We affirm that the work was completed by the author(s) listed in this article, and all will be responsible for any liabilities relating to the content of this article.

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