Phytochemical and Pharmacological Screening of Seeds and Fruits Pulp of *Cucurbita moschata* Duchesne Cultivated in Egypt

Naglaa Z.H. Eleiwa
Pharmacology Dept., Faculty of Veterinary Medicine, Zagazig Univ., Egypt

Riham Omar Bakr
Pharmacognosy Dept., Faculty of Pharmacy, October Univ. For Modern Sciences and Arts, Egypt

Shaza A. Mohamed
Pharmacognosy Dept., Faculty of Pharmacy (Girls), AL-Azhar Univ., Egypt

Corresponding author E-mail : nag_zakaria@yahoo.com

ABSTRACT

Family Cucurbitaceae is one of the most important families known for its edible fruits. It was, therefore, deemed of interest to carry out phytochemical and pharmacological studies on one of its species which is *Cucurbita moschata* Duchesne as one of widely used edible plants. Phytochemical screening of its seeds and fruits pulp revealed the presence of carbohydrates, saponins and steroids in considerable amounts in both of them. GLC analysis for lipoidal content of seeds revealed that lauric acid was the major saturated fatty acid while oleic and linoleic acids were the major unsaturated fatty acids. Vitamin A and E were estimated using RP-HPLC analysis. The highest vitamin E concentration was found in the petroleum ether extract (PEE) of the seeds while the highest vitamin A concentration was observed in 70% aqueous methanolic extract (AME) of seeds. Pharmacological investigation revealed that, AME of *Cucurbita moschata* Duchesne seeds and fruits pulp have favorable anti-inflammatory activities without notable ulcerogenic effect; that usually associated the anti-inflammatory drugs; based on their antioxidant properties. Moreover, there are remarkable analgesic and antidepressant activities recorded for AME of its seeds.

Keywords- *Cucurbita moschata* Duchesne, lipid profile, vitamins, analgesic, anti-inflammatory, antidepressant.

1. INTRODUCTION

Edible fruits have played a crucial role in the diet of people. Family Cucurbitaceae, commonly referred to as "Gourd, pumpkin or melon family" is a famous family which contains different economically important species of edible and medicinally useful plants [1]. Cucurbits are among the largest and most diverse plant families, cultivated worldwide in a variety of environmental conditions. The fruits of cucurbits are very useful in terms of human health, i.e. purification of blood, removal of constipation, good for digestion and give energy. Seeds or fruit parts of some cucurbits are reported to possess purgatives, emetics and anthelmintic properties due to the secondary metabolite cucurbitacins content [2]. The family contains 130 genera and about 800 species [3]. It extends its roots deep in history where its fruits were represented graphically on the walls of the Ancient Egyptian tombs and preserved specimens are still present in the Egyptian and European museums [1].

One of the important genus belonging to this family is *Cucurbita* [4].Genus *Cucurbita* comprises 27 species widely spread in Asia, Europe and America. It includes species grown for their fruit and edible seeds (squashes, pumpkins and marrows), as well as some species grown only as gourds [5,6].

There are three economically important *Cucurbita* species, namely *Cucurbita pepo* L.,*Cucurbita maxima* and *Cucurbita moschata* Duchesne, which have different climatic adaptations and are widely distributed in agricultural regions worldwide [7].

Cucurbita moschata Duchesne is a monoecious, having both male and female flowers on the same plant. These bright and colorful flowers have extremely short life spans and may only open for as short a time as one day [8].The leaves of *C. moschata* are light green in color, simple, alternate, broadly ovate, about 23cm long and 28cm wide, roughly serrate, palmately lobed, highly pubescent and hairs forming a cushion on the adaxial surface [9].

The fruit of *C. moschata* also known as calabaza. The color of pumpkins is derived from the orange pigments abundant in them. The main nutrients are lutein and both alpha and beta carotene, the latter of which generates vitamin A in the body [8].

Pumpkin seeds are considered as a balanced source of good proteins. They are very nourishing and energizing. Pumpkin seeds, being rich in zinc content, aid the healing process. Other nutrients including magnesium, phosphorus, copper, potassium, niacin, folic acid, riboflavin, and thiamin are also present. Trigonelline and nicotinic acid, isolated from pumpkin paste caused significant reductions in blood glucose, cholesterol and triglycerides, indicating improvement in the diabetic condition [10].

Pumpkin has also antioxidant activity and is considered as a natural source of lutein, selenium, β-carotene, vitamin E and vitamin C [11].The seeds contain phytosterols which may be responsible for shrinking of the enlarged prostate gland in addition to many phenolic acids [12,13].

The aim of this study is to determine the lipid profile of *C.moschata* seeds and to screen the main constituents, evaluate vitamin A and E contents and investigate some pharmacological activities of its seeds and fruits pulp**.**

2. MATERIALS AND METHODS

2.1Plant Material

C.moschata was bought from local markets in Cairo, Egypt. Authentication of the plant was performed by Dr. Therese Labib Youssef (Consultant of plant taxonomy, Ministry of Agriculture, Egypt).

2.2 Standards and Reagents:

Solvents; Methanol, petroleum ether, chloroform (HPLC grade, Merck**)**,*Chemicals*;α-naphthol, hydrochloric acid, sodium hydroxide, ammonia solution , sulphuric acid , glacial acetic acid, picric acid (analytical grade, BDH),mercuric chloride and potassium iodide. *Standard drugs*;carragenan(carragenan kappa-type III) , diclofenac sodium, indomethacin, imipramine hydrochloride and doxorubicin.

2.3 Animals

Mature male albino rats and mice weighing 150–200 g and 20–25 g respectively were used in this study. All experimental animals were provided from the Experimental Lab., Animal Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. Animals were fed laboratory diet and water *ad libitum*. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC).

2.4 Plant Extraction

C.moschata seeds were separated from the fruits pulp and cleaned. Seeds and fruits pulp were dried to constant weight at 50°C in a drying oven then were powdered. The ground seeds and fruits pulp were extracted with 200 mL petroleum ether using Soxhlet apparatus for 6 hrs on water bath according to AOAC Official Method [14]. After extraction, the excess of the solvent was removed under reduced pressure using a rotary vacuum evaporator, yielding petroleum ether extract (PEE). Residual air dried powders of seeds and fruits pulp were further extracted with 70%aqueous methanol. The excess of the solvent was distilled off using rotary evaporator at 50°C, yielding aqueous methanolic extract (AME). Both PEE and AME were kept in refrigerator for the current study.

2.5 Phytochemical Screening

Identification of the major chemical constituents of seeds and fruits pulp was carried out using the chemical tests on the powdered seeds and fruits pulp using standard procedures [15,16,17].

2.6 Estimation of Vitamins A and E by Reversed-Phase HPLC

Vitamins A and E were estimated by RP-HPLC where conditions were as follows: HPLC system Knauer; column: RP-C18, 5um (150 mm x 4 mm) (separation, Czech Republic); mobile phase: methanol-water (9:1, v/v); eluent flow rate: 1.5 ml/min; Knauer pump, detection: UV at 295 nm (Knauer); injection volume: 20 µl of the samples and standard vitamins in ethanol [18].

2.7 Determination of Lipoidal Matter of *C. moschata* Seeds

2.7.1 Preparation of unsaponifiable matter (UNSAP)

One gram of PEE was saponified by refluxing with ethanolic KOH (20%) at 60°C for 2 hrs. The cooled reaction mixture was diluted with an equal volume of distilled water and exhaustively extracted with ether. The combined ethereal extract was washed several times with distilled water till free from alkalinity and dehydrated over anhydrous sodium sulphate. After evaporation of ether to dryness, the residue was kept for GLC analysis [19].

2.7.2 Preparation of saponifiable matter (SAP) and fatty acid methyl esters (FAME)

The saponified extract was freed from their potassium salts by acidification with HCl (5N) to liberate the fatty acids which were extracted several times with ether (3 x 15 mL). The combined ethereal extract was washed several times with distilled water till free of acidity, and dried over anhydrous sodium sulphate. The washed combined ethereal extract was evaporated to dryness. The residue, as well as the standard fatty acids were dissolved in 10 mL of anhydrous methanol and methylated by drop-wise addition of MeOH: H_2SO_4 (50:3). The reaction mixture was refluxed for 3 hrs (3 times). Reacting mixture was extracted with ether; ethereal extract was collected then evaporated on rotary evaporator at 50°C till dryness. Two drops of double distilled chloroform solution were added to dissolve the FAME and the solution was kept in a desiccator for GLC analysis [20].

Table (1): Conditions for GLC analysis of (FAME) and (UNSAP) of *C. moschata* Seeds.

GLC	Conditions	
	FAME	**UNSAP**
Column type	70% thermo TR-FAME	Methyl phenyl silicone
Column dimensions	30m × 0.25 mm i.d.	1.5m × 4 mm i.d.
Temperature programming	Increased 70º C to 190C at 8/mn.	Increased from 70º C to 270 C at 10/mn
Carrier gas	Nitrogen	Nitrogen
Flow rate	30 ml/min	30 ml/min
Sample size	1 µl	1 µl
Injector temperature	200°C	250° C
Detector temperature	300°C	300°C

2.7.3 GLC analysis of UNSAP and FAME

Ether extract of the UNSAP and FAME were analyzed by GLC technique against the available authentics, adopting the condition mentioned in Table (1). Identification of the hydrocarbons, sterols, triterpenoids and fatty acid methyl esters was carried out by comparing retention times of the peaks with those of the available authentics. The qualitative estimation of each peak was achieved by using a computer integrator, adopting the internal normalization procedures.

2.8 Analgesic Activity Evaluation

The hot plate method of Jacob and Bosovski[21]was used to evaluate the analgesic activity. Mature male albino mice weighing 20-25 gm were classified into 4 groups; each of five. The first group was left as control and injected intra-peritoneally (i.p.) with the solvent (sterile saline solution) while the second group was injected i.p. with diclofenac sodium at a dose of 10mg/kg b wt [22].The 3rd and 4thgroups were injected i.p. with AME of *C.moschata* fruits pulp and seeds respectively at a dose of 448 mg/kg b. wt[23,24]. Ten minutes later, each mouse was placed in a two litres-beaker immersed in water bath thermostatically controlled at 56^{o}C.The time elapsed till the mouse licks its paw or jumps was considered as the reaction time and was taken as a measure of analgesic effect. Readings were taken at 10, 20, 30, 60, 90 and 120 minutes post treatment.

2.9Anti-inflammatory Activity Evaluation

Rat hind paw edema method by [25] was applied to determine the anti-inflammatory activity of AME of *C. moschata* fruits pulp and seeds using diclofenac sodium as a standard. Mature male albino rats weighing 150–200 g were used. The animals were divided into 4 equal groups ;each of five as previously mentioned in the analgesic evaluation. After 1 hr, edema in the right hind paw was induced by injecting 0.1 mL of 10% carragenan. The thickness of the paw was measured using a skin caliber 1, 2, 3, and 4 hr. after the carragenan injection to determine the anti-inflammatory activity of the tested extracts.

2.10 UlcerogenicActivity

AME of *C.moschata* fruits pulp and seeds were tested for their ulcerogenic activity using diclofenac sodium and indomethacin as reference drugs according to method described by [26].Male albino rats weighing 150-200 g were fasted for 12 hrs prior to drug administration and water was given *ad libitum*. The animals were divided into five equal groups; each of eight. The first group was received 1% gum acacia (suspending vehicle) orally once a day and was left as a control while the second group was received diclofenac sodium at a dose of 10 mg/kg/day orally [22]. The third group was received indomethacin at a dose of 10 mg/kg/day orally [27]**.** The 4thand 5thgroups were received AME of *C. moschata* fruits pulp and seeds respectively at a dose of 448 mg/kg/day orally [23, 24]. The drugs were administered orally once a day for three successive days. Animals were sacrificed by over dosage of ether after 6 hrs from the last dose. The stomach was removed, opened along the greater curvature and examined for ulceration. The number and severity of discrete areas of damage in the glandular mucosa were scored .The ulcer score was calculated according to the 1 to 5 scoring system devised by [26] as follows:

Score

1: 1 or 2 minute sporadic punctate lesions
2: Several small lesions
3: One extensive lesion or multiple moderate-sized lesions
4: Several large lesions
5: Several large lesions with stomach perforation

Stomach ulceration was expressed in terms of ulcer index (U.I.)

Ulcer index (U.I.) = Mean ulcer score of a group of animals similarly treated × % of ulcerated animals of this group [28].

2.11 Antidepressant Activity

2.11.1Forced Swim Pool Test (FST)

Forced swim pool method (FST) described by [29] was followed. Mice were classified into 4 groups, each of 5 and then injected i.p with solvent (control group), reference drug imipramine hydrochloride; 32mg/kg b.w.[30] (2nd group) and AME of *C.moschata* fruits pulp and seeds at a dose of 448 mg/kg (3rd& 4th groups respectively) [23,24]30 min. before the test session. Two swim sessions were conducted; an initial 15min pre-test followed by a 5 min test 24 hrs later. The animals were placed in a chamber (diameter: 45 cm: height: 20 cm) containing water up to height of 15 cm at 25 ± 2 °C. The period of immobility (passive floating without struggling and making only those movements which are necessary to keep its head above the surface of water) during the 5 min. test period was measured and recorded representing the depressant mood of the animal.

2.11.2Tail Suspension Test (TST)

Tail suspension test (TST) was performed according to the method described by [30] .Mice were classified into 4 groups as those mentioned in the (FST). 30 min. later, adhesive tape around the animal's tail was wrapped in a constant position and a suspension hook was passed through the adhesive tape as close as possible to the tail to ensure the animal hangs with its tail in a straight line. The animals were observed continuously for 6 min. and the time spent immobile was calculated for each mouse and recorded. In a manner analogous to the forced swim test, immobility is reduced by a wide variety of antidepressants so, used as an indicator in the antidepressants' activities evaluation.

2.12 Oxidant/Antioxidant Markers

Rats were randomly allocated into 4 groups, each of 8 rats asfollow:1stgroup served as a control, 2ndgroup received doxorubicin as oxidative stress inducer by 2.5 mg/kg b.wt, i.p., the 3rd and 4th groups received AME of *C. moschata* fruits pulp and seeds respectively at a dose of 448 mg/kg

orally once daily over a period of 2 weeks before doxorubicin treatment[23,24].After 2 weeks from the starting of the experiment, rats of all groups except group (1) were i.p administered doxorubicin at a dose of 2.5 mg/kg b.wt every other day for2 weeks [31]**.** At the end of the experimental period, serum samples were collected from all treated rats to determine Oxidant/antioxidant markers. L- Malondialdehyde (MDA), superoxide dismutase (SOD) and catalase activity (CAT) were calorimetrically assayed according to [32,33,34] respectively**.**

3. RESULTS

3.1Phytochemical Screening

C. moschata seeds and fruits pulp were screened for the presence of carbohydrates, saponins, flavonoids, tannins alkaloids, cardiac glycosides, anthraquinones, and sterols. The results were summarized in Table (2). Results revealed the presence of carbohydrates, saponins and sterols and triterpenoids in both fruits pulp and seeds. Phenols and tannins appeared as trace amount while the flavonoids, alkaloids, cardiac glycosides and anthraquinones were absent.

Table(2): Qualitative Phytochemical Screening of Seeds and Fruits pulp of *C. moschata*.

Test	Fruits pulp	Seeds
Carbohydrates	++	++
Saponin	++	++
Flavonoids	-	-
Tannins\Phenol	±	±
Sterols\Triterpene	++	++
Cardiac glycosides	-	-
Alkaloids	-	-
Free anthraquinones\Anthraquinone glycosides	-	-

[-] absent, [++] present, ± traces

3.2 Estimation of Vitamin A and E of Seeds and Fruits Pulp of *C.moschata* by RP-HPLC

RP-HPLC analysis revealed that the highest vitamin E concentration was found in the PEE followed by AME of the seeds. In addition, the highest concentration of vitamin A was observed in the AME of the seeds followed by PEE of the fruits pulp, Table (3).

Table (3): Vitamin A and E concentration in of seeds and fruits pulp of *C. moschata*.

Concentration[µg/100g]	Seeds AME	Seeds PEE	Fruits pulp AME	Fruits pulp PEE
Vitamin A	60.18	3	16.056	52.66
Vitamin E	473.64	1234	200.62	106.66

3.3 Determination of Lipoidal Matter of *C.moschata* Seeds

GLC analysis of hydrocarbons and sterols in seeds of *C. moschata* Duchesne revealed that the percentage of total identified hydrocarbon was93.85% where heneicosane represented the highest concentration (49.68%) followed by eicosane (12.81%) and nonadecane (12.65%) while the lowest hydrocarbon was hexadecane (0.09%). As denoted in Table (4), the total identified sterol represented 3.74% where cholesterol represented the highest concentration (1.48%) followed by stigmasterol (1.22%). Percentage of total unidentified unsaponifiable matter was 2.39%.

Table (4): GLC analysis of unsaponifiable matter of *C.moschata* seeds.

Retention time (min.)	Identified compounds	Percentage %
12.717	Tetradecane,C14	0.57
14.794	Hexadecane, C16	0.09
16.262	Heptadecane, C17	0.12
17.861	Octadecane, C18	3.21
18.710	Nonadecane, C19	12.65
19.834	Eicosane, C20	12.81
20.765	Heneicosane, C21	49.68
22.645	Docosane, C22	4.008
23.420	Tricosane, C23	3.93
24.969	Pentacosane, C25	1.06
26.431	Hexacosane, C26	1.82
28.086	Octacosane, C28	3.87
% total identified hydrocarbon		93.85

31.409	Cholesterol, C27	1.48
33.377	Stigmasterol, C28	1.22
35.044	B-Sitosterol, C29	0.41
36.772	Alpha-Amyrin, C30	0.62
% total identified sterol		3.74
% unidentified unsaponifiable matter		2.39

The fatty acid profile is summarized in Table (5), which shows the percentage of individual fatty acid observed. There were seven fatty acids identified by comparison with the fatty acid methyl ester standards. Saturated fatty acids in *C. moschata* represented 89.32% .The highest saturated fatty acid was lauric acid (70.46%) followed by palmitic acid (11.68%) while the lowest saturated fatty acid was heptacosylic acid (1.13%).The unsaturated fatty acid represented 10.67% where oleic acid and linoleic acid were identified and nearly were equal (5.45 and 5.21%) respectively.

Table (5): The composition (%) of fatty acid of *C. moschata*seeds.

Retention time (min.)	**Identified compounds**	**Percentage %**
10.831	Lauric acid, C12	70.46
12.636	Myristic acid, C14	1.33
15.617	Palmitic acid, C16	11.68
18.580	Stearic acid, C18	4.71
32.184	Heptacosylic acid, C27	1.13
% Total identified saturated F.A		89.32
19.157	Oleic acid, C18:1	5.45
20.268	Linoleic acid, C18:2	5.21
% Total identified unsaturated F.A		10.67
% Total unidentified F.A		0.00001

3.4 Analgesic Activity Evaluation

Results presented in Table (6) illustrated the analgesic activity of AME of *C.moschata* fruits pulp and seeds following the intra-peritoneal (i.p.) injection at a dose level of 448 mg/kg using diclofenac sodium as a reference drug. From the obtained results it was clear that AME of *C.moschata* seeds showed analgesic activity compared to the control and diclofenac treated groups as they increase the reaction time that represent the analgesic activity while the AME of fruits pulp showed non-significant effect compared to the control.

Table (6): Analgesic activity of AME of *C.moschata* fruits pulp and seeds using Diclofenac sodium as reference drug in mice . Mean ±SE

Group	**Reaction time in seconds after**					
	10 min.	**20 min.**	**30 min.**	**60 min.**	**90 min.**	**120 min.**
Control	11.40± 0.24^{c}	12.40±0.74^{c}	12.60±.67^{c}	11.80±.37^{c}	11.40±.50^{c}	12.00±.54^{c}
Diclofenac	37.20± 0.91^{a}	45.60±0.92^{a}	55.40±1.4^{a}	61.80±1.7^{a}	65.40±1.2^{a}	73.60±.67^{a}
Fruits pulp AME	12.00± 0.83^{c}	12.20±0.58^{c}	12.80±.96^{c}	12.40±.74^{c}	12.00±.70^{c}	12.40±.50^{c}
Seeds AME	24.80± 1.3^{b}	33.00±1.3^{b}	40.40±1.6^{b}	44.00±1.7^{b}	44.60±1.2^{b}	44.40±.40^{b}

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

3.5 Anti-inflammatory Activity Evaluation

The intradermal injection of carragenan (10%) at a dose of 0.1 ml in the rat paw of the hind limb significantly increased its thickness after 1, 2, 3 and 4 hours post injection. Likewise, the i.p. injection of AME of *C.moschata* fruits pulp and seeds at a dose of 448 mg/kg significantly decreased the thickness of rat paw after one hour till the end of the experiment compared with the control and diclofenac treated rats as shown in Table (7).

Table (7): Effect of AME of *C.moschata* fruits pulp& seeds and diclofenac sodium on rat hind-paw thickness at different time intervals after induction of edema using carrageenan. (Mean ±S.E)

Group	Initial thickness	Thickness of rat paw (mm) after			
	Zero time	1 hr.	2 hr.	3 hr.	4 hr.
Control	0.18±.014[a]	0.58±0.02[a]	0.77±0.02[a]	0.92±.01[a]	1.04±0.046[a]
Diclofenac	0.18±.008[a]	0.27±0.01[d]	0.33±0.01[d]	0.37±.01[d]	0.45±0.022[d]
Fruits pulp AME	0.21±.009[a]	0.47±0.02[b]	0.57±0.01[b]	0.63±.02[b]	0.76±0.029[b]
Seeds AME	0.19±.004[a]	0.33±0.02[c]	0.43±0.01[c]	0.50±.01[c]	0.61±0.018[c]

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

3.6 UlcerogenicActivity

AME of *C.moschata* fruits pulp and seeds which exhibited marked anti-inflammatory activity were tested for their ulcerogenic activity.Diclofenac and indomethacin were used as reference drugs. Results presented in Table (8) revealed that, AME of *C.moschata* fruits pulp and seeds at a dose of 448 mg/kg elicited significant decrease in the ulcer score, index and incidence of gastric ulceration compared with the reference drugs. It is clear that; the antiulcerogenic effect of seeds extract was excellent, reaching the control level.

Table (8):Ulcerogenic activity of AME of *C. moschata* fruits pulp and seeds, Diclofenac sodium and indomethacin in rats. (Mean ± S.E)

Ulcerogenic activity			
Group	**Mean ulcer score**	**Incidence of gastric ulceration**	**Ulcer index**
Control	0.00±0.00	0.00%	0.00
Diclofenac	0.80±0.02[b]	60%	48
Indomethacin	3.40±0.20[a]	100%	340
Fruits pulpAME	0.20±0.01[c]	20%	4
Seeds AME	0.00±0.00	0.00%	0.00

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

3.7 Antidepressant Activity

Antidepressant effect of AME of *C.moschata* fruits pulp and seeds using imipramine hydrochloride as reference drug in mice that present in Table (9) displayed that, rats treated with seeds extracts showed significant decrease in their immobilization time ;that represents the antidepressant activity; in both tests (forced swim pool test (FST) and tail suspension test (TST) compared with the control and imipramine treated rats while those treated with AME of fruits pulp showed non-significant effect compared with the control.

Table (9):Antidepressant effect (Forced Swim Pool Test (FST), Tail Suspension Test(TST) of AME of *C.moschata* fruits pulp and seeds & imipramine hydrochloride in mice. (Mean ± S.E)

Groups	Immobility time (seconds)	
	FST	TST
Control	126.60±2.65[a]	150.60±2.61[a]
Imipramine HCL	64.20±1.42[c]	82.20±1.24[c]
Fruits pulp AME	125.20±2.26[a]	148.00±1.87[a]
Seeds AME	88.60±1.02[b]	109.40±2.52[b]

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

3.8 Oxidant/Antioxidant Markers

Our results showed that, administration of doxorubicin to rats significantly decreased serum catalase (CAT) and superoxide dismutase (SOD) and significantly increased serum malondialdehyde (MDA) concentrations compared to the control group. It was observed that, the pre-treatment with AME of *C.moschata* fruits pulp and seeds guard against the decreases in the activity of such enzymes and the increases of MDA concentrations and the best results were belonged to the group pretreated with AME of fruits pulp, Table (10).

Table (10): Effect of AME of *C.moschata*fruits pulp and seeds on SOD, CAT activities and MDA concentrations in rats treated with doxorubicin. (Mean ± S.E)

Groups	MDA(nmol/ml)	SOD (unit/L)	CAT (nmol/ml)
Control	31.02±0.37^{d}	1.22±0.08^{a}	1.59±0.03^{a}
Doxorubicin only	78.02±0.96^{a}	0.61±0.04^{d}	0.82±0.01^{c}
Fruits pulp AME +doxorubicin	45.62±0.30^{c}	0.98±0.03^{b}	1.28±0.06^{b}
Seeds AME +doxorubicin	52.83±1.79^{b}	0.77±0.04^{cd}	1.18±0.08^{b}

Means with the same column carrying different superscripts are significant at ($p < 0.05$).

4. DISCUSSION

Use of plants as a source of medicine has been inherited and is an important component of the health care system due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine [35].

Numerous evidences have shown that increased consumption of fruits and vegetables reduce the risk of various pathological events such as cancer, cardiovascular and cerebrovascular diseases [36].This is often attributed to the antioxidants content of the fruits and vegetables such as Vitamin C, E, carotenoids and flavonoids that prevent damages caused by free radicals [37]. Many researchers have paid attention toward the Cucurbitaceae family. The seeds and fruits of plants belonged to this family such as *Citrullus colocynthis* and *Momordica chirantia* have been evaluated for their antioxidant, anti-inflammatory and analgesic activities [38,39,40].

In our study , phytochemical and pharmacological studies have been performed on seeds and fruits pulp of one of the most popular member of Cucurbitaceae family cultivated in Egypt ; *C.moschata*.

C. moschata is characterized by a wide variability in its bioactive constituents as well as the biological activities. The variability in these active constituents present in the plant extract depend on the method of extraction and type of solvent employed [41].

In the current work, *C. moschata* seeds and fruits pulp were screened for the presence of different classes of active constituents and the results revealed that carbohydrates, saponins, sterols and triterpenoids represent the major identified classes.

C. moschata has been known for its richness in vitamins A and E but their estimation in the Egyptian variety has not been performed before, therefore vitamins A and E were evaluated in PEE and AME of seeds and fruits pulp of *C. moschata* using RP- HPLC analysis. The results revealed that, *C. moschata* seeds represent a rich source of vitamins with predominance of vitamin E in the PEE (1.234 mg/100g) and predominance of vitamin A in the AME (60.18 µg/100g).

Fatty acid composition and lipid content was estimated using GLC analysis. Lauric and palmitic acid were the major saturated fatty acid while the major identified unsaturated fatty acids were oleic and linoleic acid.

Cholesterol represented the highest identified sterol (1.48%) followed by stigmasterol (1.22%).

On similar ground, *Eromosele and Eromosele* [42]reported that the seeds contained linoleic acid, omega-6 fatty acid (5.21%), an essential fatty acid conferring to nutritional value. Linoleic acid is important for its metabolic role in the synthesis of prostaglandins [43].Korean *C. moschata* showed higher oleic acid (31.34%) and linoleic acid (35.72%) compared with 5.45% and 5.21% respectively in the Egyptian variety [44].

The earlier phytochemical investigations on *C.moschata* suggested the presence of active compounds such as mono-unsaturated fatty acids (MUFA) like oleic acid (18:1), amino acids as tryptophan and glutamate, anti-oxidants as vitamin E, B-complex group of vitamins such as thiamine, riboflavin, pantothenic acid, vitamin B6 (pyridoxine) and folate, essential minerals like copper, manganese, potassium, calcium, iron, magnesium, zinc, and selenium [45].

Seeds and fruits pulp AME of *C. moschata* were further screened for their analgesic activity. Hot plate test is a well validated model for detection of opiate like analgesic drugs where the pain response is from spinal origin [46].

Our results revealed that, AME of seeds showed remarkable analgesic activity while the AME fruits pulp extract showed non-significant effect compared to the control.

Evidences stemmed from animal studies suggested that pumpkin-seed extract helps to reduce inflammation and works as an analgesic. The researchers found that the mice showed fewer pain-related behaviors, such as tail flicking, with pumpkin-seed extract than with morphine [47].

Generally, the plants are known to contain some steroidal substances responsible for the relieve of pain with immunomodulatory and antioxidative properties which has been reported in earlier studies .These substances tend to assist in the reduction of pain through the stimulation of the immune system and the reduction of prostaglandins that are responsible for the pain[48].

In addition, Pumpkin seeds contain glutamate, which is required in the synthesis of gamma-amino butyric acid (GABA), an anti-stress neurochemical that helps reduce anxiety, irritability, and induce a state of analgesia [45].

Evaluating the anti-inflammatory activity compared with diclofenac elicited that, both seeds and fruits pulp AME exerted significant anti-inflammatory activities compared with the control that associated with no ulcerogenic (seeds AME) or mild ulcerogenic; 20% (fruits pulp AME) properties.

Previous studies revealed that, the addition of pumpkin seeds to the diet has compared favorably with use of the non-steroidal anti-inflammatory drug indomethacin in reducing inflammatory symptoms. Unlike indomethacin, pumpkin seeds do not increase the level of damaged fats (lipid peroxides) in the linings of the joints, a side-effect that actually contributes to the progression of arthritis [47].

This anti-inflammatory effect could be explained by *Craig et al.* [45] who reported that, the constituents tocopherol " vitamin E " (recorded by high level in the RP-HPLC analysis of seeds in our study) and selenium may have protective functions towards the oxidative degradation of lipids, vitamins, hormones, and enzymes.

On the same ground, pretreatment of *C.pepo* prevented the inflammatory action of Carrageenan by decreasing prostaglandins E2 level, which may be due to the presence of vitamin E and C in the flesh of *C. pepo* [49].

Another school of thought stated that, oleic acid (the highest % of MUFA recorded in our study) could be reported as an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells [50]. Several studies have demonstrated that unsaturated fatty acids, including oleic acid, can inhibit both secretion and activity of phospholipase A_2 and arachidonic acid release and thus subsequent products from its metabolism are inhibited [51,52].

It is well known and reported that, carrageenan brings about inflammation by the release of mediators of inflammation (prostaglandins, histamine, bradykinin, leukotrienes…etc) and also several free radicals are released during such inflammation [53].

In fact, antioxidants are compounds that protect cells from free radicals. Free radicals, although being natural by-products of cellular metabolism, can attach to healthy cells, leading to disease in the body [54].

In our study, both seeds and fruits pulp AME showed significant free radicals scavenging activity by guarding against the decreases in the activity of antioxidant enzymes (CAT & SOD) and the increases of MDA concentrations as a response to oxidative stress induced by doxorubicin, so this antioxidant properties could be responsible for the reduction of inflammation documented in our findings.

AME of fruits pulp and seeds have a very high concentration of the antioxidant vitamin E (documented in our work).The constituents tocopherol, vitamin A and selenium have a protective function towards the oxidative degradation of lipids, vitamins, hormones, and enzymes[55].

Bavec et al. [56] reported that the raw pumpkin offers 8567 IU of the anti-oxidant vitamin A, which is 171 percent above the recommended daily allowance for vitamin A as established by the USDA.

Previous report recorded that pumpkin seeds are very rich in manganese (4543 mg per 100 grams; about 198% of daily-recommended intake). Manganese is an important co-factor for the antioxidant enzyme, superoxide dismutase, which helps the body resist infection and eliminate free radicals [45].

Regarding the antidepressant activity, our study proved the significant antidepressant activity of the seeds AME while the fruits pulp AME showed non- significant changes. The antidepressant activity may be attributed to its reported tryptophan content [57].

Pumpkins are rich in tryptophan and volunteers, who took part in a study, were able to cope with pressure better after taking this substance found in pumpkin seeds. Those patients also experienced higher levels of mental well-being. [58].

In addition, pumpkin seeds are also known for their high contents of zinc, which can help the brain to convert tryptophan into serotonin which creates feelings of well-being [59].

5. CONCLUSION

Based on our findings, it could be concluded that seed of *C. moschata* is considered as a rich source of vitamins A and E as well as, it contains favorable percentage of unsaturated fatty acids ;mainly oleic and linoleic acids. In addition, AME of *C. moschata* seeds and fruits pulp could be represented as anti-inflammatory candidate without notable ulcerogenic effect; that usually associated the anti-inflammatory drugs; based on their antioxidant properties. Moreover, there are remarkable analgesic and antidepressant activities recorded for AME of its seeds.

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