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Kumquat As A Potent Natural Material To Improve Lipid Profile Of Hypercholestrolemic Rats

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ABSTRACT

Different kumquat fruit portions (whole, peel and pulp) were analyzed for its chemical constituent, essential oil content, total phenolic and total flavonoids contents then added in different levels (2.5 and 5%) to thirty five albino hypercholesterolemic rats diets aiming to improve their lipid profile. By the end of the experiment (28 days) TC, TG, TL, LDL, HDL, atherogenic indices (AC, CRR, AI) were analysed. The different kumquat portions (whole, peel and pulp) contained 77.24%, 72.68% and 77.23% moisture, 6.01%, 3.66% and 7.68% protein, 12.21%, 8.45% and 7.36% crude fat, 25.57%, 24.26% and 22.04% fiber, 3.17%, 2.97% and 3.43% ash and 53.04%, 60.66% and 59.49% carbohydrate respectively. Interestingly, Kumquat pulp had the highest total phenolic content (0.406mg GAE g^{-1} DW) and whole kumquat had the highest flavonoid content (0.0068 mg QE g^{-1} DW).The principal constituents of the essential oil were α-Myrcene (4.68%), Ocimenyl acetate (3.01%) and Limonene (80.63%) which was the most abundant compound. The total lipid, total cholesterol and LDL ratios were improved by 27.51%, 28.25% and 40.77% compared with the positive control group. Significant correlations were observed among the levels of serum triglyceride, serum total cholesterol, serum total lipid, high-density lipoprotein (HDL) cholesterol and atherogenic indices (AC, CRR, AI), implying that hypocholesterolemic effects of 5% whole kumquat were partly attributed to the reduced absorption of lipid and cholesterol.

Keywords: *Kumquat – Hypercholesterolemia - Total Phenolics – Total Flavonoids – Essential Oils Compounds.*

INTRODUCTION

Hypercholesterolemia is a common clinical metabolic and/or genetic disorder that promotes functional and structural vascular wall injury (Napoli and Lerman, 2001). The World Health Report estimates that worldwide about 8% of all disease burdens in developed countries is caused by raised cholesterol levels in excess of the theoretical minimum, 3.8mmol/L (WHO, 2002).The underlying mechanisms for these deleterious effects involve a local inflammatory response and release of cytokines and growth factors (Napoli and Lerman, 2001).Total cholesterol levels above 200 mg/dl have repeatedly been correlated as an independent risk factor for development of peripheral vascular (PVD) and coronary artery disease (CAD) (Stapleton *et al*., 2010), thus, caused about 46% of death reasons in Egypt (WHO, 2014). Some hypercholesterolemia treatment options that have become widely used, including pharmaceutical therapies which can decrease circulating cholesterol by preventing either its formation in

the liver or its absorption in the intestine, also have pleiotropic effects witch can directly improve peripheral vascular outcomes (Stapleton *et al*., 2010). Moreover, drugs are not available over the counter and cannot be used for general health maintenance. Thus, increasing interests have drawn towards safe alternative products derived from natural bioresources (Thilakarathna and Rupasinghe, 2012). A lot work has been carried out by researches on various plants to evident their effectiveness in hypercholesterolemia (Harikumar *et al*., 2013). Kumquat (*Fortunella margarita* Swingle) is the smallest of the true citrus fruits belonging to the family Rutaceae. The flesh is sour, and the fruit is eaten together with the peel. Kumquats are also an excellent source of nutrients and phytochemicals, including ascorbic acid, carotenoids, flavonoids and essential oils (Wang *et al*., 2012). Kumquats are suitable for various products and they can be eaten raw, as whole fruit or added to beverages (Peng *et al*., 2013). Ji-lie *et al*., (2008) found that the flavonoid extracts in the kumquats can efficiently lower the blood lipid in the obese rats with hyperlipidemia. Lien *et al*., (2009) indicated that the daily repeated oral administration of kumquat peel extracts decreased the level of total cholesterol, triacylglycerol and LDL. However, a few studies on the effect of kumquat on blood lipid profile have been shown. The present study is designed to evaluate the possible beneficial effect of kumquat portions (whole, peel, pulp) on lipid profile parameters in induced hypercholesterolemic rats.

MATERIAL AND METHODS

Plant material:

Kumquats (*Fortunella margarita*) were purchased from a local market (Shiben El-Kom, El-Minofia, Egypt). It was washed under running water, separated into three portions, whole kumquat, kumquat peel and kumquat pulp, then it was dried at 40˚C in an air draught oven (Plue Pard Drying Oven, Taiwan) and grinded to a fine powder and kept in dark glass bottle in deep freezer (-16˚C) for further analysis.

Chemical composition:

The samples were analysed for chemical composition (moisture, protein, fat, carbohydrates and ash) using the AOAC procedures (A.O.A.C. 2012). The extraction procedure used for the determination of total phenols and total flavonoids was adapted from Chun *et al*., (2003) ; Franke *et al*., (2004)

Determination of total phenolics:

Total phenolic content was determined by the Folin–Ciocalteu micro-method (Saeedeh and Asna, 2007). A 20 µL aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu's reagent followed by 300 µL of 200 g L^{-1} Na_2CO_3 solution. The mixture was incubated in a shaking incubator at 40˚C for 30min and its absorbance at 760 nm was measured. Gallic acid was used as standard for the calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$A = 0.98C + 9.925 \times 10^{-3}$ ($R^2 = 0.9996$)

Where A is the absorbance and C is the concentration (mg GAE g^{-1} dry weight).

Determination of total flavonoids:

Total flavonoid content was determined by the method of Ordon *et al*., (2006). A 0.5mL aliquot of 20 g L^{-1} $AlCl_3$ ethanolic solution was added to 0.5 mL of extract solution. After 1 h at room temperature, the absorbance at 420 nm was measured. A yellow colour indicated the presence of flavonoids. Extract samples were evaluated at a final concentration of 0.1 mg mL^{-1}. Total flavonoid content expressed as quercetin equivalent (QE) was calculated using the following equation based on the calibration curve:

$y = 0.0255x$ ($R^2 = 0.9812$)

Where x is the absorbance and y is the concentration (mg QE g^{-1} DW).

Essential oil steam distillation:

Whole fruits (300 g) were homogenized for 2 min with 300mL of bidistilled water and placed into a 5 L round-bottom flask. The homogenate was steam-distilled for 2 h to obtain the essential

oils. Samples were then stored in a dark glass bottle at -16°c for further analysis (Board, 2003).

Fractionation of essential oils by GC–MS:
The sample of essential oil (5 µL) was diluted in *n*-hexane (0.5 mL) and analyzed with the Thermo GC-MSD (Trace DSQ II, Thermo Fisher Corporation, USA) apparatus equipped with a TR-5 capillary column (30 m × 0.25 mm internal diameter, 0.25 µm film thickness). Helium (1.0 mL/min) was used as a carrier gas. The injector and the transfer line were kept at 250 ˚C and 280˚C, respectively. The column was maintained at 60˚C for 4 min and then programmed to rise to 240˚C at 4˚C/min and held for 15 min at 240˚C. The MS was operated in the EI mode at 70 eV and in the *m*/*z* range 40–500. Retention indices of the separated compounds on the TR-5 capillary column were determined on the basis of a homologous series of *n*-alkanes (C9–C27). The compounds of essential oil were identified on the basis of comparison of their retention indices and mass spectra with published data and computer matching with National Institute of Standards and Technology (NIST, 3.0) libraries provided with a computer controlling the GC-MS system. The relative proportions of the essential oil constituents were expressed as percentages obtained by peak area normalization, and all relative response factors were taken as one (Adams, 2007).

Experimental animals:
Fourty adult male albino rats, six weeks old and weighing 150±30gm each at the beginning of the experiment, were obtained from the research Institute of Opthalmology, Medical Analysis, Department Giza, Egypt. Rats were housed in wire cages under normal laboratory conditions and were fed on standard diet for one week as an adaption period. Diet was introduced to rats in special food cups to avoid scattering of food. Also, water was provided to rats by glass tubes projecting through the wire cages from an inverted bottle supported to one side of the cage. Food and water were provided ad-labitum and checked daily. Standard diet was prepared from fine ingredients according to AIN, (1993).

Experimental groups:
Male albino rats were divided randomly into two main groups, the first, negative control group (normal) (n= 5), fed on basal diet and the second group (hypercholesterolemic) (n=35), was fed on diet containing cholesterol (1.5%) and bile salts (0.25%) for 21 consecutive days to achieve hypercholesterolemia.

Then, the hypercholesterolemic group (n=35) was divided into seven groups, 5 rats each. First group (positive control group) was fed on basal diet, the second, third and fourth groups were fed on 2.5% dried kumquat portions (whole, peel, and pulp), respectively and the fifth, sixth and seventh groups were fed on 5% dried kumquat portions (whole, peel and pulp), respectively for 4 weeks.

Blood collection:
At the end of the experimental rats were starved for 12 h then Blood samples were collected into clean centrifuge tubes, set at room temperature for 15 minutes, put into a refrigerator for one hour, centrifuged (Centurion Scientific K3 Series, Centurion Scientific LTD, England) at 3000 rpm for 10 minutes to separate serum. Serum was carefully separated and transferred into clean dry Wassermann tubes using Pasteur pipette and then kept frozen at -2˚C till analysis.

Biochemical assays:
The serum triglycerides (TG), high density lipoprotein (HDL), total cholesterol (TC) and total lipids (TL) were determined according to the methods described by Fossati and Prencipe, (1982) ; Demacker *et al*., (1980) ; Richmound, (1973) ; Covaci *et al*., (2006), respectively. The determination of low density lipoprotein cholesterol (LDLc) and very low density lipoprotein cholesterol (VLDLc) were carried out according to the method of Lee and Nieman, (1972). Atherogenic indices [(cardiac risk ratio (CRR), atherogenic coefficient (AC) and atherogenic index (AI)] were determined according to (Casterlli and Levitar, 1977 ; Kikuchi-Hayakawa *et al.*, 1998 ; Dobia´sˇova´ and Frohlich, 2001).

Statistical analysis:
All experiments were performed in five replicates. The data were recorded as means ± standard deviations and analyzed with SPSS (version 12.0 for Windows, SPSS Inc., 223 South Wacker Drive, Chicago, USA). Differences were considered significant at $P < 0.05$ (Keppel, 1991).

RESULTS AND DISCUSSION

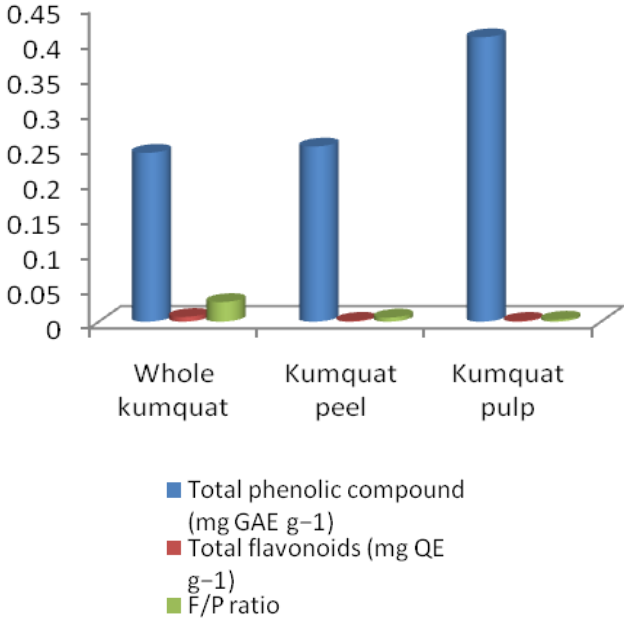
The proximate chemical compositions of dry kumquat portions (whole, peel, and pulp) are presented in table (1). Whole kumquat contained 77.24% moisture, 6.01% protein, 12.21% fat, 25.57% fiber, 53.04% carbohydrate and 3.17% ash. USDA, (2014) results were similar to the results obtained for whole kumquat. As shown in table (1) Dried Peel of kumquat contained 72.68% moisture, 3.66% protein, 8.45% fat, 24.26% fiber, 60.66% carbohydrate and 2.79% ash. In dried kumquat pulp, the chemical composition was 77.23% moisture, 7.68% protein, 7.36% fat, 22.04% fiber, 59.49% carbohydrate and 3.43 % ash.

Table-1. Chemical composition of kumquat fruits portions (on dry weight basis).

Parameters %	Whole	Peel	Pulp
Moisture	77.24	72.68	77.23
Protein	6.01	3.66	7.68
Fat	12.21	8.45	7.36
Fiber	25.57	24.26	22.04
Ash	3.17	2.97	3.43
Carbohydrate	53.04	60.66	59.49

The proximate total phenolic and flavonoids compounds of whole dried kumquat, dried kumquat peel and dried kumquat pulp were presented in Fig (1). Whole dried kumquat contained 0.241 mg GAE g^{-1} phenolic compounds. According to the result of Wang *et al*., (2007) total polyphenols in kumquat was 52.3 mg/gdb (gallic acid equivalents). Also, the amount of the total phenolic compounds in dried kumquat peel was 0.250 mg GAE g^{-1}. This result was in disagreeing with the given result by Sadek *et al*., (2009). The total phenolic levels in this study are much higher than those measured in grapefruit, mandarin, lemon and sweet orange (Li *et al*., 2006). Figure (1) also showed that dried kumquat pulp contained 0.406 mg GAE g^{-1} phenolic compounds. However, this result was higher than the result obtained by Ramful *et al*., (2011). Interestingly, kumquat pulp had a high total phenolic amount compared to whole kumquat and kumquat peel. It was detected by another study (Ramful *et al*., 2011) that total phenolic compound was high in some citrus peel extracts (orange, clementine, mandarin, tangor, tangelo and pamplemousses).

Figure-1. Total phenolic and total flavonoid compounds in dried kumquat portions (whole, peel and pulp).



Total flavonoids in whole dry kumquat were 0.0068mg QE g^{-1}. However, the total flavonoids were found to be lower than those reported by Wang *et al*., (2007). Dried kumquat peel contained 0.0013 QE g^{-1} flavonoids. Another study by Wang *et al*., (2008) reported that total flavonoids in dried kumquat (C. Microcarpa) peels were 41.0mg/g, db (rutin equivalents). The given results in Fig (1) also showed the total amount of flavonoids in dried kumquat pulp which were 0.0012 QE g^{-1}. This difference between the obtained results and the previous results (Wang *et al*., 2007 ; Wang *et al*., 2008 ; Sadek *et al*., 2009 ; Ramful *et al*., 2011) might

be due to different environmental conditions. The flavonoid-phenolic ratio was calculated to show the importance of flavonoids in total phenolic content. The F/P ratios were 0.028 in dried whole kumquat, 0.0059 in dried kumquat peel and 0.0029 in dried kumquat pulp. Dried whole kumquat had the highest F/P ratio compared to the other portions (peel and pulp). Figure (1) assessed the phenolic, flavonoid acid-rich extract of kumquat (*F.margarita*), one of the plant foods, for its beneficial effect on lowering plasma lipid profile (Table 3,4,5,6) as maintained by another studies (Pal *et al*., 2003 ; Afonso *et al*., 2013)**.**

Table (2): Volatile Components Identified in Whole Kumquat fruits Oil.

No	Compound name	Molecular formula	Area %
Monoterpene			
1	α-Myrcene	$C_{10}H_{16}$	4.68
2	Limonene	$C_{10}H_{16}$	80.63
3	Ç-Terpinene	$C_{10}H_{16}$	0.12
4	α-Terpinene	$C_{10}H_{16}$	0.18
5	Artemiseole	$C_{10}H_{16}O$	0.11
6	α-Thujene	$C_{10}H_{16}$	0.19
7	Geranyl acetate	$C_{12}H_{20}O_2$	1.51
8	Acetic acid, decyl ester (CAS)	$C_{12}H_{24}O_2$	0.29
9	α-PINENE	$C_{10}H_{16}$	1.12
Monoterpene alcohol			
10	Linalool	$C_{10}H_{18}O$	0.29
11	α-TERPINEOL	$C_{10}H_{18}O$	0.72
12	Carveol, dihydro,cis	$C_{10}H_{18}O$	0.09
13	Ocimenyl acetate	$C_{10}H_{18}O_2$	3.01
14	Terpinen-4-ol	$C_{10}H_{18}O$	0.58
Monoterpene derivative			
15	trans-Carveol	$C_{10}H_{16}O$	0.78
16	Cis-Carveol	$C_{10}H_{16}O$	0.18
Sesquiterpenes			
17	ë-Elemene(CAS)	$C_{15}H_{24}$	0.10
18	ç-Elemene	$C_{15}H_{24}$	0.30
19	Ç-Gurjunene	$C_{15}H_{24}$	0.10
20	α-copaene	$C_{15}H_{24}$	0.11
Aliphatic aldehyde			
21	Decanal	$C_{10}H_{20}O$	0.09
Alcohol			
22	Cis-Farnesol	$C_{15}H_{26}O$	0.15
Other compounds			
23	Cyclopropane,1-isopropenyl-1-(Terahydrofuran-2,5-dion-3-yl)-	$C_{10}H_{12}O_3$	0.12
24	TRICYCLO[5.2.1.0(2,6)]DECANE,4-METHYL-	$C_{11}H_{18}$	0.16
25	NERYL ACETATE	$C_{12}H_{20}O_2$	0.17
26	7-METHYLENEBICYCLO[3.3.0]OCT AN-2-OL	$C_9H_{14}O$	0.10
27	Benzene,1-ethyl-4-methyl(CAS)	C_9H_{12}	2.50
28	TRICYCLO[3.2.1.0(2,8)]OCTAN-7-ONE, 6-METHYL-6-(2-METHYL-2-PROPEN-1-L)-	$C_{13}H_{18}O$	0.15
29	Calarene	$C_{15}H_{24}$	0.30
30	6-epi-shyobunol	$C_{15}H_{26}O$	0.11
31	1,1,4,7-TETRAMETHYLDECAHYDRO -1H-CYCLOPROPA[E]AZULEN-4-OL#	$C_{15}H_{26}O$	0.51
32	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-HEXADECAMETHYLOCTASILOXANE #	$C_{16}H_{50}O_7Si_8$	0.16
33	Eicosane (CAS)	$C_{20}H_{42}$	0.14
34	BENZENE,METHYL(1-METHYLETHENYL)-	$C_{10}H_{12}$	0.11
35	Geraniol formate (CAS)	$C_{11}H_{18}O_2$	0.14

The detected constituents from steam distilled oil of whole kumquat are listed in table (2), together with their weight percentages and their molecular formula, thirty five components, representing 100% of the total oil, were identified by GC-MS analysis. The oil contained (35 compounds) 9 Monoterpenes, 5 Monoterpenes alcohols, 2 Monoterpene derivative, 4 Sesquiterpenes, 1 Aliphatic aldehyde, 1 Alcohol and 13 other compounds. Monoterpens were the characteristic and the most abundant constituents, as in other citrus fruits. The essential oil is characterized by a large presence of monoterpenes (88.83%). Limonene (80.63%) was the most abundant compound, followed by α-myrcene (4.68%), Geranyl acetate (1.51%), α-Pinene (1.12%), Acetic acid, decyl ester (0.29%). α-Thujene , α-Terpinene ,Ç-Terpinene , and Artemiseole occurred at <0.1% in the essential oil of kumquat fruits. In a comparison of our data with the

Table-3. Effect of adding 2.5 or 5% levels of Kumquat fruits portions (whole, peel and pulp) on serum lipid profile in hypercholesterolemic rats.

Groups parameters	Negative control group	Positive control group	2.5% kumquat level			5% kumquat level			LSD
			Whole	Peel	Pulp	Whole	Peel	Pulp	
TL (mg/dl)	189.4^{h} ±2.29	313.57^{a} ± 1.99	259.05^{d} ± 1.88	243^{f}± 2.42	274.95^{b}± 1.82	227.46^{g}± 0.37	249.92^{e}± 2.62	270.44^{c}± 1.59	2.55
TG (mg/dl)	57.8^{h}± 0.83	84.8^{a}± 0.83	70.2^{d}± 0.83	66.2^{f}±0 .83	73.4^{c}± 0.54	62.4^{g}± 0.54	68.2^{e}± 0.83	75.2^{b}± 0.0.83	0.99
TC (mg/dl)	99.16^{h}±1 .92	177.96^{a} ±1.55	146.64^{d} ±1.75	137.04^{f} ±1.8	158.42^{b}± 1.35	127.68^{g}± 0.98	140.84^{e}± 1.58	152.04^{c}± 1.41	2.02

*Data presented are in means ± standard deviations for five replicates in each group. Values in the same row not sharing a common superscript letter differ significantly at $P \leq 0.05$.

results of Bernhard and Scrubis, (1961); Koyasako and Bernhard, (1983) ; Umano *et al*., (1994); Choi, (2005) ; Schirra *et al*., (2008) ; Wang *et al*., (2012) ; Peng *et al*., (2013) we found that monoterpenes were the most abundant volatile compounds in kumquat oils in all these reports, and limonene and myrcene were the most common components. The limonene content of kumquat (*F. margarita)* oil was comparatively less than that reported for kumquat (*F. japonica)* peel oil (Choi, 2005), kumquat *(F. crassifolia)* peel oil (Wang *et al*., 2012), and kumquat (*Fortunella margarita)* Swingle oil (Peng *et al*., 2013). Previous studies have shown that d-limonene inhibits lipid peroxidation and prevents free radical-induced damage (Devi *et al*., 2004).The á-myrcene content of the essential oil was higher than that reported for kumquat (*F. japonica)* peel oil and oval kumquat (Choi, 2005 ; Peng *et al*., 2013), and was lower than that reported for kumquat (*F. crassifolia)* peel oil (Wang *et al*., 2012).The essential oil contained five monoterpene alcohols (4.69%): linalool (0.29%), α-Terpineol (0.72%), Carveol, dihydro, cis (0.09%), Ocimenyl acetate (3.01%), and Terpinen-4-ol (0.58%). The linalool content of the essential oil was higher than that reported in kumquat *(F. japonica)* (Choi, 2005), similar to that reported for kumquat (*F. crassifolia)* peel oil (Wang *et al*., 2012), and less than that in oval kumquat (Peng *et al*., 2013). The Terpinen-4-ol content was high, as with other Fortunella species oils (choi, 2005 ; Peng *et al*., 2013).

The monoterpenes derivative contents of the oil presented here were much higher than those of kumquat *F. japonica* peel (Choi, 2005).The amount of Sesquiterpenes were detected to be 0.61% in this oil, with the four main compounds being: ë-Elemene (0.10%), ç-Elemene (0.30%), Ç-Gurjunene (0.10%) and α-copaene (0.11%).The Aliphatic aldehyde content of the oil presented here were less than those of oval kumquat (Peng *et al*., 2005). In the study of Choi, (2005) Cis-Farnesol was detected in traces compared with the obtained result from table (2). The last 13 compounds were about 4.67% in the essential oil. Interestingly, the given results are consistent which those of Umano *et al*., (1994) ; Choi, (2005) ; Wang *et al*., (2012) ; Peng *et al*., (2013) who identified similar components in the essential oils of kumquats.

Table (3) revealed the effect of adding 2.5 or 5% levels of kumquat fruits portions (whole, peel and pulp) on serum lipid profile in hypercholesterolemic rats.

Hypercholesterolemic group administrated with 5% whole kumquat represented the highest ($p \leq 0.05$) improvement percentage in serum total lipids (27.46%) compared to positive control group followed by 2.5% kumquat peel, 5% kumquat peel, 2.5%whole kumquat group, 5% kumquat pulp group, and 2.5% kumquat pulp group which were improved by 22.5%, 20.29%, 17.38%, 13.75%, and 12.31%, respectively compared to control positive group.

Table-4. Effect of adding 2.5% or 5% levels of kumquat fruits portions (whole, peel and pulp) on LDL, HDL and VLDL in hypercholesterolemic rats.

Group parameters	Negative control group (normal)	Positive control group	2.5% kumquat level			5% kumquat level			LSD
			Whole	Peel	Pulp	Whole	Peel	Pulp	
LDL (mg/dl)	45.8^{h}± 0.83	144.2^{a}± 0.83	108.4^{d}± 1.14	95.6^{f}± 1.14	121.4^{b}± 1.51	85.4^{g}± 0.54	101^{e}±1	116^{c}±1.22	1.37
HDL (mg/dl)	41.8^{a}± 1.3	16.8^{h}± 0.83	24.2^{e}± 0.83	28.2^{c}± 0.83	22.6^{f}± 0.54	29.8^{b}± 0.83	26.2^{d}± 0.83	21.2^{g}± 0.83	1.13
VLDL (mg/dl)	11.56^{h}± 0.16	16.96^{a}± 0.16	14.04^{d}± 0.16	13.24^{f}± 0.16	14.44^{c}± 0.16	12.48^{g}± 0.1	13.64^{e}± 0.16	14.84^{b}± 0.16	0.2

*Data presented are in means ± standard deviations for five replicates in each group. Values in the same row not sharing a common superscript letter differ significantly at P ≤ 0.05.

Data from table (3) indicated that the levels of serum triglycerides significantly ($p < 0.05$) decreased in 5% whole kumquat group, 2.5% kumquat peel group and 5% kumquat peel group by 26.41%, 21.93% and 19.57%, respectively compared to positive control group. Reduced level of triglyceride in serum may help lower the risk of coronary heart disease (Davignon and Cohn, 1996). These results are in agreement with reports of other workers (Ji-lie *et al*., 2008 ; Lien *et al*., 2009). Meanwhile, the highest ($p \leq 0.05$) improvement in serum total cholesterol occurred when the hypercholesterolemic rats fed on 5% whole kumquat (127.68±0.98 mg/dl) while, the lowest ($p \leq 0.05$) improvement was observed in the group fed on 2.5% kumquat pulp (158.42±1.35 mg/dl) compared to positive control group (177.96±1.55 mg/dl). Similar results in lowering TC values were reported by Ji-lie *et al.,* (2008) ; Lien *et al*., (2009). From the given results in figure (1) and table (2) , whole kumquat is a good source of phenolic compounds, flavonoids and essential oils. Phenolic compounds and flavonoids exhibited a potent hypercholesterolemia capacity as it was described by Pal *et al*., (2003) ; Afonso *et al*., (2013).

Data in Table (4) summarized the effect of adding 2.5% or 5% levels of kumquat portions (whole, peel and pulp) on LDL, HDL and VLDL in hypercholesterolemic rats.

The results demonstrated that the levels of low density lipoprotein (LDL), and very low density lipoprotein (VLDL) were decreased in hypercholesterolemic groups administrated with kumquat portions (whole, peel and pulp) compared to positive control group, while high density lipoprotein (HDL) elevated.

More considerable reduction ($p \leq 0.05$) in LDL was observed in rats administrated with 5% whole kumquat (85.4± 0.54 mg/dl) compared to positive control group (144.2±0.83 mg/dl) followed by 2.5% kumquat peel, 5% kumquat peel, 2.5% whole kumquat which were 95.6±1.14 mg/dl, 101±1 mg/dl, 108.4±1.14 mg/dl, respectively. The mean value of HDL in the group fed on 5% whole kumquat was significantly higher ($p < 0.05$) than that in positive control group followed by 2.5% kumquat peel group (28.2±0.83 mg/dl), 5% kumquat peel group (26.2±0.83 mg/dl), 2.5% whole kumquat group (24.2±0.83 mg/dl), respectively. Lien *et al*., (2009) demonstrated that blood HDL-C increased in obese mice when treated with different extract fractions from kumquat (*Fortunella japonica)* peels. The best values of VLDL in hypercholesterolemic groups were for 5% whole kumquat group (12.48±0.1 mg/dl) which showed a significant ($p \leq 0.05$) decrease compared to positive control group (16.96±0.16 mg/dl), meanwhile the lowest improvement occurred in hypercholesterolemic group fed on 5% kumquat pulp (14.84±0.16 mg/dl). These results are in agreement with reports of other studies (Ji-lie *et al*., 2008 ; Lien *et al*., 2009). In this study, 5% whole kumquat group increased plasma HDL cholesterol levels

Table-5. Effect of adding 2.5% or 5% levels of kumquat fruits portions (whole, peel and pulp) on HDL/TC Ratio (HTR %) and LDL/HDL Ratio (LHR %) in hypercholesterolemic rats.

Groups Parameters	Negative Control (normal)	Positive control group	2.5% kumquat level			5% kumquat level			LSD
			Whole	Peel	Pulp	whole	peel	Pulp	
HTR* (mg/dl)	42.14^{a}±0.59	9.43^{g}±0.39	16.49^{e}±0.39	20.57^{c}±0.39	14.26^{f}±0.37	23.33^{b}±0.5	18.59^{d}±0.45	13.93^{f}±0.5	0.58
LHR* (mg/dl)	1.09^{g}±0.02	8.59^{a}±0.38	4.48^{c}±0.12	3.38^{e}±0.08	5.37^{b}±0.17	2.86^{f}±0.07	3.85^{d}±0.11	5.47^{b}±0.22	0.23

•Data presented are in means ± standard deviations for five replicates in each group. Values in the same row not sharing a common superscript letter differ significantly at P ≤ 0.05.
*HTR = HDL/TC Ratio, LHR= LDL/HDL Ratio.

Table-6. Effect of adding 2.5% or 5% levels of kumquat fruits portions (whole, peel and pulp) on the atherogenic indices (AC= TC-HDL/HDL, CRR= TC/HDL, AI= Log TG/HDL) in hypercholesterolemic rats.

Groups Parameters	Negative Control (normal)	Positive control group	2.5% kumquat level			5% kumquat level			LSD
			Whole	Peel	Pulp	whole	peel	Pulp	
AC* (mg/dl)	1.37^{g}±0.03	9.6^{a}±0.43	5.06^{c}±0.14	3.85^{e}±0.09	6.01^{b}±0.18	3.28^{f}±0.08	4.37^{d}±0.12	6.17^{b}±0.25	0.26
CRR* (mg/dl)	2.37^{g}±0.03	10.6^{a}±0.43	6.06^{c}±0.14	4.85^{e}±0.09	7.01^{b}±0.18	4.28^{f}±0.08	5.37^{d}±0.12	7.17^{b}±0.25	0.26
AI* (mg/dl)	0.24^{d}±0.23	0.64^{a}±0.14	0.46b^{c}±0.01	0.36^{bcd}±0.01	0.5^{abc}±0.01	0.31^{cd}±0.01	0.36^{bcd}±0.09	0.54^{ab}±0.02	0.13

*Data presented are in means ± standard deviations for five replicates in each group. Values in the same row not sharing a common superscript letter differ significantly at P ≤ 0.05.
*AC = TC-HDL/HDL, CRR =TC/HDL, AI= Log (TG/HDL).

and decreased plasma LDL cholesterol levels (Table 5), an effect that may again be mediated by the flavonoids; since according to Middleton *et al.*, (2000) ; Ji-lie *et al.*, (2008). A high consumption of phenolic compounds has already been found to decrease serum cholesterol and triglyceride concentration in rats (Hirose *et al.*, 1991 ; Afef *et al.*, 2000). Some studies have shown promise, proof that increasing HDL-C levels confers a reduction in major cardiovascular outcomes independent of changes in levels of low-density lipoprotein cholesterol or triglycerides has been more elusive (Singh *et al.*, 2007).

Data recorded in table (5) shows the effect of adding 2.5% or 5% levels of kumquat fruits portions (whole, peel and pulp) on HDL/TC ratio (HTR %) and LDL/HDL ratio (LHR %) in hypercholesterolemic rats.

Generally, all treatments improved the HTR and LHR so that it reached the high level characteristic for the healthy rats. The best treatment that improved HTR and LHR in the hypercholesterolemic groups was observed in the diet supplemented with 5% whole kumquat for 28 consecutive. The phenolic and flavonoid compounds have been known to exhibit hypocholesterolemic properties through the regulation of hepatic gene expression related to lipid metabolism (Ji-lie *et al.*, 2008 ; Jung *et al.*, 2012). Therefore, phenolic compounds, as well as flavonoids in figure (1) could be involved in the lipid-lowering effects of the whole kumquat. There was a negative correlation between the HDL: TC (HTR %) ratio and the risk of coronary heart disease (Malaspina *et al.*, 1981 ; Barter and Rye, 1996 and Rajendra Chary & Estari Mamidala, 2013).

Table (6) showed the effect of adding 2.5% or 5% levels of kumquat fruits portions (whole, peel and pulp) on the atherogenic indices (AC, CRR, AI) in hypercholesterolemic rats.

The level of atherogenic coefficient (AC) was improved by 65.83%, 59.89% and 54.47%, respectively when the hypercholesterolemic rats fed on 5% whole kumquat, 2.5% kumquat peel and 5% kumquat peel, compared to positive control group. The best hypercholesterolemic group in cardiac risk ratio (CRR) was that administrated with 5% whole kumquat (4.28±0.08 mg/dl) and the lowest improvement was in 5% kumquat pulp group (6.17±0.25 mg/dl) compared to positive control group (9.6±0.43 mg/dl).

All kumquat portions (whole, peel and pulp) treatment produced a decrease in the AI especially by adding 5% whole kumquat compared to positive control group and the other hypercholesterolemic groups, followed by 5% kumquat peel then 2.5 % kumquat peel which were 0.36±0.09 mg/dl, 0.36±0.01 mg/dl, respectively. Atherogenic indices are powerful indicators of the risk of heart disease the higher the value, the higher the risk of developing CVD and vice versa (Usoro *et al.*, 2006 and Krishna Gopal, 2013). The obtained results have suggested the anti-atherogenic potential of whole kumquat, its peel and pulp portions. In this study, the whole kumquat possesses a hypocholesterolemic activity, an effect that may again be mediated by the flavonoids and phenolic compounds (Fig 1); since according to (Lee *et al.*, 2010 ; Jung *et al*., 2012).

CONCLUSION

In conclusion, kumquat portions (whole, peel and pulp) were efficient in the protection against hypercholesterolemia by decreasing serum TC, TG, TI, and LDL-C and increasing HDL-C, and thus decreasing the atherogenic indices (AC, CRR, AI). Notably, the studied kumquat could be considered as potential sources of flavonoids, phenolic compounds and essential oil pattern which could be used in a wide variety of applications, mainly in the food and confectionary industries. The pharmaceutical, cosmetic and perfume industries are other possible outlets of these flavonoids, phenolic compounds and essential oils.

REFRENCES

1. Adams, R.P (2007). Identification of essential oil components by gas chromatography/Quadrupole Mass Spectroscopy; Allured Publishing Corporation: Carol Stream, IL, USA.
2. Afef K.E., F. Jan, R. Alexander, and T. Siv (2000). Effects of dietary phenolic compounds on tocopherol, cholesterol and fatty acids in rats. Lipids, pp. 427–435.
3. AIN (1993). Purified diet for laboratory rodent: Final Report. American Institute of Nutrition .J. Nutrition, 123:1939-1951.
4. AOAC International Official Methods of Analysis (2012). 19th ed., Gaithersburg, MD: AOAC International.
5. Barter, P. J., and Rye, K. A. (1996). High density lipoproteins and Coronary Heart Disease. Atherosclerosis, 121, 1–12.
6. Bernhard, R. A., and Scrubis, B (1961). The isolation and examination of the essential oil of the kumquat. Journal of Chromatography, 5, 137–141.
7. Board, N (2003). The complete technology book of essential oils (aromatic chemicals). s.l.:Asia Pacific Business Press Inc.
8. Casterelli, T. and Levitar, Y (1977). Atherogenic Index. Curr. Presc., P 39.
9. Choi, H. S (2005). Characteristic odor components of kumquat (Fortunella japonica Swingle) peel oil. Journal of Agricultural and Food Chemistry, 53, 1642–1647.
10. Chun, O.K., Kim, D.-O., Moon, H.Y., Kang, H.G. and Lee, C.Y (2003). Contribution of individual polyphenolics to total antioxidant capacity of plums. J. Agric. Food Chem. 51, 7240–7245.
11. Covaci A, Voorspoels S, Thomsen C, van Bavel B and Neels H (2006). Evaluation of Total Lipids Using Enzymatic Methods for the Normalization of Persistent Organic Pollutant Levels in Serum. Science of The Total Environment, Volume 366, Issue 1, Pages 361–366.
12. Davignon, J., and J. S. Cohn (1996). Triglycerides: a risk factor for coronary heart disease. Atherosclerosis. 124 (Suppl.): 57–64.
13. Demacker, p. M.; Von-janssen, H.E;Hifman, A.M (1980). Vants lear, A. and jansen, A.P. Measurment of high density lipoprotein

cholesterol in serum. Comparison of six isolation methods combined with enzymatic cholesterol analysis.Clin.Chem.26:1780-1789.
14. Dobia´sˇova Milada´, and Jiri Frohlich (2001). The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FERHDL). Clinical Biochemistry Volume 34, Issue 7, Pages 583–588.
15. Fossati, P. and Prencipe,I (1982). Serum triglycrides determination colorimetrically with an enzyme that produce hydrogen peroxide. Clin.Chem.28:2077-2083.
16. Franke, A.A., Custer, L.J., Arakaki, C and Murphy, S.P (2004). Vitamin C and flavonoid levels of fruits and vegetables consumed in Hawaii. Journal of Food Composition and Analysis 17, 1–35.
17. Harikumar K., S. Abdul Althaf, B. Kishore kumar, M. Ramunaik, and CH. Suvarna A (2013). Review on Hyperlipidemic.International Journal of Novel Trends in Pharmaceutical Sciences 59:71.
18. Hirose, N., Inoue, T., Nishihara, K., Sugano, M., Akimoto, K.,Shimizu, S., and Yamada, H (1991). Inhibition of Cholesterol Absorption and Synthesis in Rats by Sesamin. J. Lipid Res. 32,629–638.
19. Ji-lie LI, Wang Wei, Zeng Chao-zhen, Liu Zong-min and LI Zhong-hai (2008). Effects of Kumquat Flavonoid Extracts on the Blood Lipid Reduction of Obese Rats with Hyperlipidemia. Journal of Central South University of Forestry & Technology; 05.
20. Jing Li ,Yu Zhang, Shengjie Fan, Ming Gu,Yu Guan, Xiong Lu, Cheng Huang, and Zhiqin Zhou (2013). Preventive and ameliorating effects of citrus d-limonene on dyslipidemia and hyperglycemia in mice with high-fat diet-induced obesity. European Journal of Pharmacology.Volume 715, Issues 1–3, Pages 46–55.
21. Jung, C. H., Cho, I., Ahn, J., Jeon, T.-I., and Ha, T.-Y (2012). Quercetin reduces high-fat diet-induced fat accumulation in the liver by regulating lipid metabolism genes. Phytotherapy Research.
22. Keppel Geoffrey (1991). Design and Analysis: A Researchers Handbook 3rd.
23. Kikuchi-Hayakawa H., N. Onodera, S. Matsubara, E. Yasuda, Y. Shimakawa and F. Ishikawa (1998). Effects of soya milk and Bifidobacterium-fermented soya milk on plasma and liver lipids, and faecal steroids in hamsters fed on a cholesterol-free or cholesterol-enriched diet.British Journal of Nutrition, Volume 79, Issue 01, pp 97-105.
24. Koyasako, A., and Bernhard, R. A (1983). Volatile constituents of the essential oil of kumquat. Journal of Food Science, 48, 1807–1812.
25. Krishna Gopal Rao. (2013). Hypoglycemic activity of extracts from *Elytraria acaulis* L. Leaves in alloxan-induced diabetic rats. 1(1):11-16.
26. Lee Jeong-Sun , Song-Hae Bok, Seon-Min Jeon, Hye-Jin Kim, Kyung-Min Do, Yong-Bok Park, and Myung-Sook Choi (2010). Antihyperlipidemic effects of buckwheat leaf and flower in rats fed a high-fat diet. Food Chemistry, Pages 235–240.
27. Lee, R. and Nieman, D (1996). Nutritional assessment. 2nd Ed., Mosby, Missouri, USA.
28. Li, S., Lo, C.Y., and Ho, C.T (2006), Hydroxylated polymethoxyflavones and methyl flavonoids in sweet orange (Citrus sinensis) flavedo. J. Agric. Food Chem. 54, 4176–4185.
29. Lien Do Ngoc, Nguyen Thuy Quynh, Nguyen Hoang Quang, Do Van Phuc1and Nguyen Thi Thanh Ngan (2009). Anti-Obesity and Body Weight Reducing Effect of Fortunella japonica Peel Extract Fractions in Experimentally Obese Mice. KKU Sci. J.37 (Supplement) 96-104.
30. Malaspina, J. P., Bussiere, H., and Calve, G. L (1981). The total cholesterol/HDL cholesterol ratio: a suitable atherogenesis index. Atherosclerosis, 40, 373–375.
31. Middleton, E. Jr., Kandaswami, C. and Theoharides, T.C (2000). The Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease and Cancer. Pharmacol. Rev., 673-751.
32. Milessa S. Afonso,Renata P.A. Bombo, Roberta M. Machado, Maria Silvia Ferrari avrador, Valéria S. Nunes, Eder R. Quintão, Marcia Koike, Sergio Catanozi, Chin Jia in, Edna R. Nakandakare, and Ana Maria Lottenberg (2013). Dietary phytosterol does not accumulate in the arterial wall and prevents atherosclerosis of LDLr-KO mice, Atherosclerosis, Pages 442–447.
33. Napoli .C and Lerman. LO (2001). Involvement of oxidation-sensitive mechanisms in the cardiovascular effects of hypercholesterolemia. Mayo Clin Proc. 76 (6):619-31.
34. Ordon JD, Gomez MA and Vattuone MI (2006). Antioxidant activities of Sechium edule (Jacq.) Swartz extracts. Food Chem 97:452–458.
35. Pal S, Ho N, Santos C, Dubois P, Mamo J, Croft K, and Allister E (2003). Red wine polyphenolics increase LDL receptor expression and activity

and suppress the secretion of ApoB100 from human HepG2 cells. J Nutr 133: 700.
36. Peng, I. Sheu, M, Lin, L; Wud, C; Chiang ,H; Lin, W; Lee, M and Chen, H (2013). Effect of heat treatments on the essential oils of kumquat (Fortunella margarita Swingle), Food Chemistry 532–537.
37. Phoebe .A. Stapleton, Adam. G. Goodwill, Milinda. E. James, Robert. W. Brock and Jefferson. C. Frisbee (2010). Hypercholesterolemia and microvascular dysfunction: interventional strategies, Journal of Inflammation. pp, 1:10.
38. Rajendra Chary Vijayagiri, Estari Mamidala. (2013). Preliminary phytochemical and in vitro anti-diabetic activity of Ficus racemosa (L.) stems bark extract. Online International Interdisciplinary Research Journal, Vol-III, Nov 2013 Special Issue. 134-141.
39. Ramful Deena, Evelyne Tarnus, Okezie I. Aruoma, Emmanuel Bourdon and Theeshan Bahorun (2011). Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps. Food Research International 44, 2088–2099.
40. Richmound, W (1973). Preparation and properties of cholesterol oxidas from Nacadia sp. And its application to enzymatic assay of total cholesterol in serum .Clin. Chem. 19:1350.
41. Sadek Samih Engy, Dimitris P. Makris and Panagiotis Kefalas (2009). Polyphenolic Composition and Antioxidant Characteristics of Kumquat (Fortunella margarita) Peel Fractions Plant Foods. Hum Nutr 64:297–302.
42. Saeedeh A and Asna U (2007). Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. Food Chem 102:1233–1240.
43. Schirra, M., Palma, A., D'Aquino, S., Angioni, A., Minello, E. V., and Melis, M (2008). Influence of postharvest hot water treatment on nutritional and functional properties of kumquat (Fortunella japonica Lour. Swingle Cv. Ovale) fruit. Journal of Agricultural and Food Chemistry, 56, 455–460.
44. Singh I.M, M.H. Shishehbor, and B.J. Ansell (2007). High-density lipoproteins a therapeutic target. JAMA, 298 pp. 786–798.
45. Thilakarathna S.H. and Rupasinghe H. P. V. (2012). Anti-atherosclerotic effects of fruit bioactive compounds: A review of current scientific evidence. Canadian Journal of Plant Science, pp. 407–419.
46. U.S. Department of Agriculture (2014). Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 27. Nutrient Data Laboratory.
47. Umano, K., Hagi, Y., Tamura, T., Shoji, A., and Shibamoto, T (1994). Identification of volatile compounds isolated from round kumquat (Fortunella japonica Swing). Journal of Agricultural and Food Chemistry, 42, 1888–1890.
48. Usoro, C.A.O., Adikwuru, C.C., Usoro, I.N. and Nsonwu, A.C (2006)."Lipid Profile of Postmenopausal Women in Calabar, Nigeria". Pak. J. Nutr. 5:79-82.
49. Wang, Y. ; Zeng, W. ; Xu ,P;Lan,Y;Zhu,R. ; Zhong,K. ; Huang,Y and Gao,H (2012). Chemical Composition and Antimicrobial Activity of the Essential Oil of Kumquat (Fortunella crassifolia Swingle) Peel. Int. J. Mol. Sci., 3382-3393.
50. Wang,Y.C ; Chuang,Y.C and Hsu,H.W (2008). The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. Food Chemistry, 277–284.
51. Wang,Y.C. ; Chuang,Y.C and Ku,Y.H (2007). Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. Food Chemistry. 1163–1171.
52. World Health Organization (2002). The World Health Report - Reducing Risks, Promoting Healthy Life.
53. World health Organization (2014). Noncommunicable Diseases (NCD) Country Profiles.

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