Chemical composition, antidiabetic, anti-inflammatory, antioxidant and toxicity activities, of the essential oil of *Fortunella margarita* peels

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Abstract

The purpose of this study was to identify the principal components of the essential oil extracted from *Fortunella margarita* peels via hydrodistillation and to evaluate in vitro its anti-diabetic, anti-inflammatory, antioxidant, and toxicity properties. Among the detected compounds were limonene, D-germacrene, β-myrcene, and α-pinene. Method of inhibiting the denaturation of Bovine Serum Albumin (BSA) was utilized to assess the anti-inflammatory properties of *Fortunella margarita*. At a concentration of 400 g/mL, a high anti-inflammatory effect was observed. The percentage of BSA protection against heat increased with increasing concentration. Also, the evaluation of antidiabetic activity by glucose uptake by yeast cells revealed that *Fortunella margarita* was more effective than the standard drug novoformine in the presence of 5 mM glucose. The antioxidant potential of the essential oil was evaluated using the DPPH free radical scavenging, reducing power and β-carotene/linoleic acid tests, where the essential oil had much lower antioxidant activity. A bioassay on the lethality of brine shrimp was conducted to determine the toxicity of the essential oil. The study reveals that the essential oil is a possible source of important bioactive compounds and that its constituents may exhibit synergistic effects. Our findings suggest that the essential oil from *Fortunella margarita* could be used in the future as a substitute for synthetic anti-diabetic, anti-inflammatory, and antioxidant agents with potential applications in the food and pharmaceutical industries.

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Introduction

Kumquats (*Fortunella margarita*) are a small, sweet and sour fruit belonging to the Rutaceae family. They are typically consumed raw as a whole fruit with the peel and without the seeds.[1,2] The nutrient and phytochemical content of kumquats includes carotenoids, ascorbic acid, flavonoids, and essential oils. Due to the presence of terpenoids and flavonoids, the peel has a palatable, sweet aroma.[3] Kumquat is a small citrus fruit and is native to Asia, specifically China and India. In Algeria, it is still an uncommonly known and consumed fruit. In some states of Algeria, kumquats are commercialized and sold as exotic fruits with high added value in large supermarkets and open markets in small towns. It is common knowledge that kumquat peels are abundant in essential oils, which play a key role in the flavour of the fruit and are widely used in the food and pharmaceutical industries.[2]

Not only are plant-derived essential oils prized for their nutritional value, but also for their functional and technical properties. Moreover, due to the presence of bioactive compounds with antioxidant properties, the quality of these fruit essential oils is enhanced.[4,5] As oxidative bursts in various cells are one of the inflammatory reactions, essential oils can also act as anti-inflammatory agents. Inflammation is a complex biological response of vascular tissues to stimuli that may be harmful. Inflammation is also associated with pain and involves, among other things, an

increase in protein denaturation, an increase in vascular permeability, and membrane alteration.[6]

Diabetes mellitus is an endocrine metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolisms, as a result of inadequate insulin secretion, insulin action, or both. Between 2017 and 2045, it is estimated that approximately 693 million people worldwide will have diabetes mellitus.[7] The majority of traditional anti-diabetic plants are, however, awaiting proper scientific and medical evaluation of their ability to improve blood glucose control and/or prevent diabetic complications.[8]

Even though they contain highly valuable bioactive molecules, *Fortunella margarita* peels are underutilized and end up as waste,[9] despite the fact that the fruit and its peel are commonly used to treat coughs, digestive issues, infections, muscle pain, and skin inflammation.[10] As far as we are aware, no study has been published evaluating the anti-diabetic, anti-inflammatory, and toxic properties of the essential oil of *Fortunella margarita* peels. The primary objective of this study is to examine the chemical composition and anti-diabetic, anti-inflammatory, antioxidant, and toxicity properties of the essential oil extracted from Algerian-grown *Fortunella margarita* peels. This study is expected to shed additional light on the pharmacological and medicinal properties of kumquat oil.

Materials and Methods

Chemicals

β-carotene, linoleic acid, Tween 40, butylated hydroxytoluene (BHT), the stable free radical 2,2-diphenyl-2-picryl-hydrazyl (DPPH•), ascorbic acid, NaOH, methanol, chloroform, potassium ferricyanide [$K_3Fe(CN)_6$], ferric chloride (FeCl3), trichloroacetic acid, diclofenac sodium and BSA were obtained from Sigma (Sigma-Aldrich, Germany).

Plant material

The fruit of *Fortunella margarita* was collected in Blida, northern Algeria (March 2021). The harvested fruit was identified and authenticated by Professor Toumi from the Department of Biological and Environmental Sciences, ENS Kouba, Algeria and confirmed by the CNCC (National Center for Control and Certification of seeds and seedlings, Algeria) where the exsiccate was deposited with registration number SRA 492/CV. To avoid damaging the oil glands, the fruits were gently peeled with a sharp knife.

Hydrodistillation extraction

Fresh peels of *Fortunella margarita* were hydrodistilled using the Clevenger. The essential oil was extracted from 500 g of peels which were added with 1 L of water into a 4 L flask for two hours. The obtained oil was separated from the water by simple decantation. This procedure does not employ any organic solvent. The obtained essential oil was stored in a brown vial, tightly closed and maintained in a cool place (4°C) away from light.

Chemical composition

Gas Chromatography/Mass Spectrometry (GC/MS)

The determination of the chemical composition of essential oil of *Fortunella margarita* peels was carried out by Gas Chromatography-Mass Spectrometry (GC/MS). A GC–FID system '7890A/5977B MSD Agilent' fitted with a fused-silica-capillary column containing a non-polar stationary phase HP5MS (30 m × 0.25 mm × 0.25 µm film thickness) was used for GC analysis. The column temperature program was 60°C for 8 minutes, then it was increased at 2°C/minute to 250°C and held at 250°C for 15 minutes. A volume of 0.2 µL was injected into the splitless GC inlet, held at 250°C. The oven temperature program was 60°C for 8 minutes, increasing at a rate of 4°C/minute to 250°C and held at 250°C for 25 minutes; the ionization mode used was electronic impact at 70 eV. The identification was established by comparison of the mass spectral fragmentation patterns with those stored in the database Adams 2017, NIST 2014 and Wiley.[7] The retention indices of the volatile extract constituents compared with those of the published index data confirmed the identification.

Evaluation of the antidiabetic activity

Glucose uptake by yeast cells

Repeated centrifugation (3,000 × g; 5 min) in distilled water was used to wash commercial baker's yeast until the supernatant fluids were clear, and a 10% (v/v) suspension was made in distilled water. Various concentrations of essential oil of *Fortunella margarita* peels (50, 100, 200, 400 µg/mL) were added to 1 mL of glucose solution 5 mM and incubated together for 10 min at 37°C. The reaction was started by adding 100 µL of yeast suspension, which was vortexed and further incubated at 37°C for 60 min. After 60 minutes, the tubes were centrifuged (2,500 × g, 5 min) and the glucose content of the supernatant was measured. All tests were done in triplicate and the absorbance was measured at 540 nm. The percent increase in glucose uptake by yeast cells was calculated using the following formula:[11]

$$\% \text{ Increase in glucose uptake} = (\text{Abs control} - \text{Abs sample})/ \text{Abs control} \times 100$$

where:
Abs control: absorbance of the control reaction.
Abs sample: absorbance of the test sample.

Evaluation of the anti-inflammatory activity

Inhibition of albumin denaturation

The protein denaturation test was carried using Gambhire *et al.*[12] method, with some changes as described by Gunathilake *et al.*[13] The reaction mixture (5 mL) which contained 0.2 mL of 1% BSA, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 0.02 mL of essential oil of *Fortunella margarita* peels, was combined and incubated in a water bath (37°C) for 15 minutes before being heated at 70°C for 5 minutes. The turbidity was measured using a spectrometer at 660 nm after cooling. The control was a phosphate buffer solution. The percentage inhibition of precipitation (protein denaturation) was calculated as a percentage of the control using the formula:

$$\% \text{ Inhibition of denaturation} = (1 - A_2/A_1) \times 100$$

where:
A_1 = absorption of the control sample.
A_2 = absorption of the test sample.

Antioxidant activity

DPPH radical scavenging activity

The DPPH (2,2-diphenyl-2-picryl-hydrazyl) free radical activity was assessed according to the procedure described by Musa *et al.*[14] Briefly, 1 mL of different concentration of essential oil of *Fortunella margarita* peels was mixed with 1 mL of ethanol solution of DPPH (0.004%). Thirty minutes later, the absorbance of each sample was measured at 517 nm against blank samples. BHT and Ascorbic acid were used as reference standards. The percentage of inhibition activity was calculated using the following equation:

$$\% \text{ Inhibition} = (Ac - As/Ac) \times 100$$

where:
Ac: absorbance of the control.
As: absorbance of the sample solution.

Reducing power

The reducing power was determined according to the procedure described by Yen and Chen.[15] Various concentrations of essential oil of *Fortunella margarita* peels were mixed with 2.50 mL of 1% potassium ferricyanide and 2.50 mL of 0.2 M sodium phosphate buffer (pH = 6.6), and incubated in a water bath (50°C for 20 min). Then, 2.50 mL of 10% trichloro acetic acid were added to the mixture that was centrifuged (3000 × g for 10 min); 2.50 mL of supernatant was then mixed with 2.50 mL distilled water and 0.50 mL of ferric chloride solution (0.1%). The reduction power of extracts was expressed as EC_{50} value. BHT and Ascorbic acid were used as reference standards and the absorbance was measured at 700 nm.

β-carotene

The test was performed consistent with the procedure described by Miraliakbari and Shahidi.[16] A mixture was prepared with β-carotene (0.5 mg) in 1 mL chloroform, 25 µL of linoleic acid and 200 mg Tween 40. The chloroform was evaporated, and 100 mL of distilled water were then added to the residue; then, 350 mL of each extract of *Fortunella margarita* peels were added to 2.5 mL of the above mixture. The test tubes were incubated in a hot water bath (50°C for 2h). The absorbance of the samples was measured at 470 nm every 30 min for 2h. The percent inhibition of the samples was calculated from the following equation:

$$I\% = [A(\beta\text{-carotene}) \text{ after 2h assay}/A \text{ initial } (\beta\text{-carotene})] \times 100$$

where:
A (β-carotene) after 2h assay: absorbance values of β-carotene remaining in the samples.
A initial (β-carotene): absorbance value of β-carotene at the beginning of the experiments.

Toxic activity

Artemia salina lethality assay

Brine shrimp (*Artemia salina*) lethality bioassay was carried out to investigate the toxicity of the essential oil. *Artemia salina* were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h.[17] After hatching, ten nauplii were drawn through a Pasteur pipette and placed in each vial containing 4.5 mL of seawater. In each experiment, 0.5 mL of various concentrations of essential oil of *Fortunella margarita* peels (1, 10, 100, 1000, 10000 µg/mL) were added to 4.5 mL of seawater and maintained at room temperature for 24h under the light and surviving larvae were counted. The assays were carried out in triplicates and percentage of mortality was determined using the equation:

$$\% \text{ Mortality} = (\text{no. of dead nauplii/initial no. of live nauplii}) \times 100$$

Statistical analysis

The mean and standard deviation (SD) of three measurements are presented.

Results

Hydrodistillation extraction

Pale yellow to colourless essential oil with strong smell of lemon was obtained from the fresh peel of *Fortunella margarita*, with percentage yield (0.3 %).

Chemical composition

Gas Chromatography/Mass Spectrometry (GC/MS)

Essential oil was obtained from the fresh peels of *Fortunella margarita* by hydro-distillation and subjected to GC-MS. The chromatographic analysis revealed the presence of 39 components, representing 98.90% of the total oil. These compounds were identified by GC-MS analysis; their retention indices and relative area percentages are shown in Table 1. The quantity of the major compound, limonene, in the essential oil obtained from *Fortunella margarita* peel is high (86.31%), followed by Germacrene D (4.67%), β-Myrcene (3.21%), α-Pinene (0.75%), Geraniolacetate (0.62%), Elixene (0.62%), trans-Carveol (0.35%), α-Terpineol (0.25%), δ-Cadinene (0.22%), 4-Terpineol (0.13%), α-Cadinol (0.13%), n-Octylacetate (0.12%), τ-Eudesmol (0.11%) and Linalol (0.1%). Sabinene, β-Pinene, τ-Terpinene, trans-p-Mentha-2,8-dienol, Viridiflorol and τ-Muurolol occurred at <0.1% in the essential oil.

Evaluation of antidiabetic activity

Glucose uptake by yeast cells

The rate of glucose transport across cell membrane in yeast cells system is presented in Figure 1. The essential oil of *Fortunella margarita* decreased the glucose movement across the

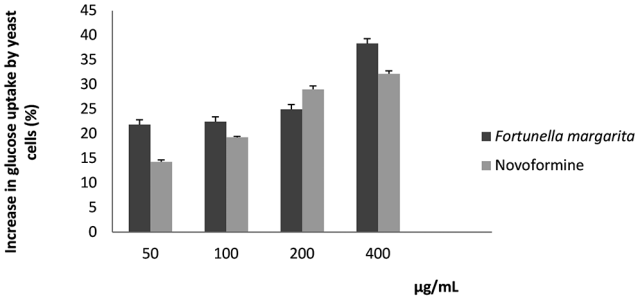

Figure 1. Effect of essential oil of *Fortunella margarita* peels on glucose uptake by yeast cells. Values are expressed as mean ± SD.

Table 1. Chemical composition of essential oil of *Fortunella margarita* peels.

No.	Compound[a]	RI[b]	RI[c]	Peak area (%)[d]	Identification method
	Monoterpenehydrocarbons			**90.54**	
1	α-Pinene	926	932	0.75	GC-MS
2	Sabinene	965	969	0.09	GC-MS
3	β-Pinene	968	974	0.08	GC-MS
4	β-Myrcene	990	994	3.21	GC-MS
5	Limonene	1036	1033	86.31	GC-MS
6	α-Ocimene	1058	1056	0.02	GC-MS
7	τ-Terpinene	1064	1065	0.05	GC-MS
8	α-Terpinolen	1089	1087	0.03	GC-MS
9	1,3,8-p-Menthatriene	1111	1111	tr	GC-MS
	Oxygenatedmonoterpenes			**1.12**	
10	Linalool	1103	1103	0.10	GC-MS
11	trans-p-Mentha-2,8-dienol	1121	1126	0.07	GC-MS
12	Limoneneoxide, cis-	1134	1134	tr	GC-MS
13	cis-p-Mentha-2,8-dienol	1136	1134	0.04	GC-MS
14	β-Erpineol	1146	1145	0.05	GC-MS
15	4-Terpineol	1176	1176	0.13	GC-MS
16	α-Terpineol	1190	1188	0.25	GC-MS
17	Safranal	1197	1201	0.03	GC-MS
18	trans-Carveol	1220	1216	0.35	GC-MS
19	cis-Carveol	1231	1229	0.06	GC-MS
20	D-Carvone	1243	1254	0.04	GC-MS
	Sesquiterpenehydrocarbons			**5.95**	
21	δ-Elemene	1336	1334	0.06	GC-MS
22	Copaene	1374	1374	0.06	GC-MS
23	β-Bourbonene	1382	1382	0.03	GC-MS
24	β-Elemene	1391	1391	0.02	GC-MS
25	trans-Caryophyllene	1416	1417	0.09	GC-MS
26	α-Caryophyllene	1451	1451	0.05	GC-MS
27	Germacrene D	1483	1482	4.67	GC-MS
28	Elixene	1495	1504	0.62	GC-MS
29	α-Muurolene	1499	1499	0.03	GC-MS
30	γ-Cadinene	1506	1513	0.04	GC-MS
31	Farnesene	1509	1509	0.06	GC-MS
32	δ-Cadinene	1523	1523	0.22	GC-MS
	Oxygenatedsesquiterpenes			**0.44**	
33	Globulol	1582	1583	0.04	GC-MS
34	Viridiflorol	1590	1590	0.08	GC-MS
35	τ-Eudesmol	1631	1632	0.11	GC-MS
36	τ-Muurolol	1642	1642	0.08	GC-MS
37	α-Cadinol	1654	1654	0.13	GC-MS
	Others			**0.74**	
38	n-Octylacetate	1215	1213	0.12	GC-MS
39	Geraniolacetate	1386	1384	0.62	GC-MS
Total volatile compounds (%)				**98.79**	
Total non-oxygenatedcompounds(%)				**96.49**	
Total oxygenatedcompounds(%)				**2.30**	

[a]Compounds identified according their families on HP-5MS column; [b]Retention indices with respect to C5–C28 n-alkanes calculated on non-polar HP5-MS capillary column; [c]Retention indices given in literature (NIST, Wiley or ADAMS on non-polar HP-MS or DB5-MS capillary column); [d]Percentage calculated from the peaks areas of GC chromatogram on non-polar HP5-MS capillary column; tr, traces.

membrane. Results also indicated that *Fortunella margarita* (38%) had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug novoformine (32%) in 5 mM glucose concentration.

Evaluation of the anti-inflammatory activity

Inhibition of albumin denaturation

Inhibition of denaturation of BSA test was used to evaluate the anti-inflammatory property of the essential oil of *Fortunella margarita* (Figure 2). The inhibition of protein (albumin) denaturation was shown to be concentration dependent in this study. The percentage of BSA protection against heat increased with increasing concentration; at the concentration of 400 µg/mL it showed a high anti-inflammatory activity (34.82%). Sodium diclofenac was used as the reference drug and it also showed a concentration dependent inhibition of protein denaturation (99.28 %).

Antioxidant activity

DPPH radical scavenging activity

DPPH is a stable free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule. It is commonly employed to study radical-scavenging activity. The presence of antioxidants in the extract decreases the DPPH free radical, which forms a purple color in methanol solution, to a yellow coloured reduced product DPPH-H by accepting an electron from the antioxidant in this assay.

The result of radical scavenging efficacy of the essential oil of *Fortunella margarita* peels compared with BHT and ascorbic acid is depicted in Figure 3. In this test, the essential oil of *Fortunella margarita* peels had much lower antioxidant activity in the DPPH test. The IC_{50} values of BHT and ascorbic acid were found to be 19.54±0.320 and 1.17±0.005 µg/mL, respectively. These results suggest that the essential oil of *Fortunella margarita* has a potency to donate electrons to reactive free radicals, converting them into more stable non-reactive species and terminating the free radical chain reaction.

Reducing power

The Ferric Reducing Antioxidant Power (FRAP) assay evaluates antioxidants ability to reduce the oxidative effects of reactive oxygen species. The assay reaction involves the reduction of Fe^{3+}/ferricyanide complex to the ferrous form Fe^{2+} with an antioxidant compound. Antioxidant activity was determined by measuring absorbance of the Perl's Prussian blue at 700 nm. This method is based on the presence of reductones, which exert the antioxidant action by breaking the free radical chain by donating a hydrogen atom.

The reducing power of the essential oil of *Fortunella margarita* peels increased with increasing concentration. The essential oil presented a low reducing power with EC_{50} value of 3.73±66.42 mg/mL, when compared to the positive controls, ascorbic acid and BHT (5.94±0.047 and 12.14±0.128 µg/mL, respectively).

β-carotene

The oxidation of linoleic acid produces hydroperoxides as free radicals during incubation at 50°C, which attack the β-carotene, resulting in a bleaching of the reaction solution. This fact is used in the antioxidant activity evaluation in comparison with ascorbic acid and BHT. In this assay, BHT had the highest antioxidant power (88.47%), followed by essential oil of *Fortunella margarita* peels (24.93%), which exhibited higher activity than ascorbic acid (10.22%), well known for its antioxidant potency (Figure 4).

Toxic activity

Artemia salina lethality assay

The brine shrimp lethality bioassay is an efficient, rapid and inexpensive test with small amount of test material being utilized and has proven to be an excellent choice for elementary toxicity investigations. Toxicity assay was conducted to determine the toxic-

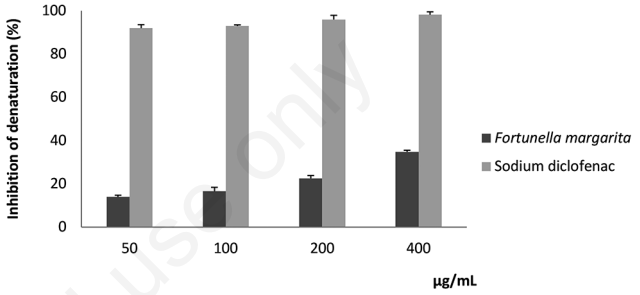

Figure 2. Effect of essential oil of *Fortunella margarita* peels on inhibition of albumin denaturation. Values are expressed as mean ± SD.

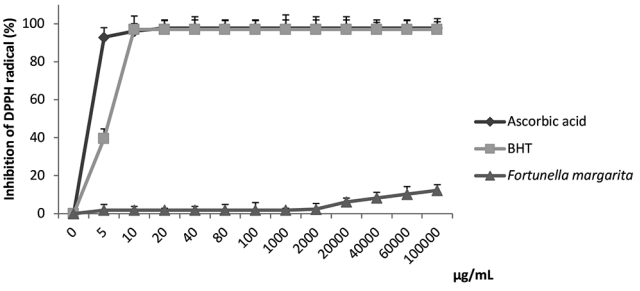

Figure 3. Free radical scavenging activity of the essential oil of *Fortunella margarita* peels. Values are expressed as mean ± SD.

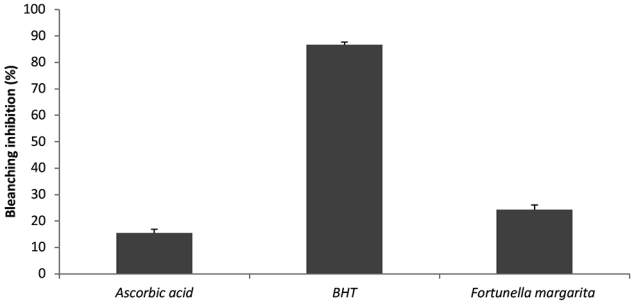

Figure 4. β-carotene bleaching activity of the essential oil of *Fortunella margarita* peels. Values are expressed as mean ± SD.

ity ability of the essential oil of *Fortunella margarita* in killing *Artemia salina* larvae which had been treated with concentrations of 1, 10, 100, 1000 and 10000 µg/mL. After 24 hours, the toxicity was determined by LC_{50} (median lethal concentration) using the probit regression analysis. Therefore, the toxicity was considered high, and LC_{50} value of *Fortunella margarita* was 70 µg/mL (Figure 5).

Discussion

The chromatographic analysis revealed the presence of the various compounds detected included limonene, D-germacrene, β-myrcene and α-pinene. Numerous reports on the chemical composition of *Fortunella margarita* peel oil have confirmed the presence of limonene as a major compound.[18] Limonene has always been the main component. For example, limonene (94.3%) was the major component of the peel oil from Argentinean *Fortunella margarita*, beside myrcene (1.9%) and geraniol (1.1%).[19] Limonene accounted for 73.4% in peel oil from China, isolated by hydro-distillation.[20] In parallel, the peel oil of *Fortunella margarita* of Californian origin contained 92.7% of limonene beside isopropyl n-propionate (1.8%) and terpinyl acetate (1.3%) isolated by a simultaneous distillation/extraction method.[21]

A previous report showed that the compositions of the fruit oils of five *Fortunella* species were strongly dominated by limonene (84.2-96.3%).[22] In another study on the characteristic odorous components in the essential oil from *Fortunella japonica* peel, limonene was the highest component, amounting to 93.73%.[23] Limonene is used to treat gastric disorders[24,25] and has an antiproliferative effect on cancer cells.[26] Antimicrobial, anti-inflammatory, sedative, and antilithic activities of limonene have also been reported.[27]

The results of the evaluation of antidiabetic activity indicated that *Fortunella margarita* had greater efficiency as compared to standard drugs. The mechanism of glucose transport across yeast cell membranes has attracted attention and is considered to be an important technique to screen various compounds/medicinal plants for their hypoglycemic activity *in vitro*. The findings showed that examined essential oil promoted the transport of glucose through yeast cells. The amount of glucose remaining in the medium after a certain time interval was used as a measure of glucose uptake by the yeast cells. According to the scientific literature, there are no reports or scientific publications reporting the evaluation of antidiabetic activity due to glucose uptake by yeast cells testing the essential oil from the peels of *Fortunella margarita*.

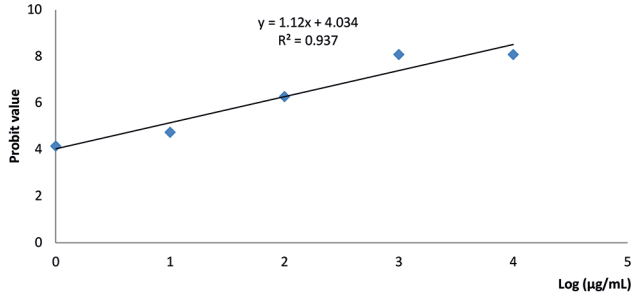

Figure 5. ***Artemia salina*** **lethality assay of the essential oil of** ***Fortunella margarita*** **peels.**

Previous research on the transport of non-metabolizable sugars and metabolizable glycosides has revealed that sugar transport across the yeast cell membrane is mediated by stereo specific membrane carriers and usually occurs via the assisted diffusion process.[28]

The present data suggest that the plant extract is capable of enhancing glucose uptake effectively, which in turn suggests that it is capable of enhancing effective utilization at 400 µg/mL concentration, thereby controlling blood glucose level as also suggested by other reports.[29]

Polyphenols are natural products well known for several notable biological properties. In the present study, the *in vitro* anti-inflammatory activity of essential oil can be attributed to its polyphenols content. The effect may be due to the synergistic effect rather than the single constituent. It has been reported that one of the characteristics of non-steroidal anti-inflammatories is their ability to stabilize heat treated albumin at physiological pH.[11,30]

The percentage of BSA protection against heat increased with increasing concentration. These results are comparable to those of Sonam[31] for the essential oil of *Citrus macroptera* where protection of the order of 26.3±1.2 at the concentration of 200 µg/mL was recorded. To our knowledge, there are no published reports of anti-inflammatory activity by inhibition of albumin denaturation. The adoption of BSA protein denaturation assay for the *in vitro* evaluation of anti-inflammatory potential of essential oil circumvented the ethical issues associated with the use of animals, particularly in early stages of screening for plants containing potential anti-inflammatory compounds.

Additionally, protein denaturation has been described as a pathological process that involves loss of configuration hence loss of functionality. This makes reduction of protein denaturation, inhibition of albumin denaturation assay, ideal for determining anti-inflammatory potential.[32]

The value of the anti-radical activity of essential oil of *Fortunella margarita* obtained is compared to the study by Eleni *et al*.[33] who found a percentage inhibition equal to 34.5±0.07% expressed at a concentration of 43 mg/mL. In another study by Nouri and Shafaghatlonbar[34] carried out on another species of kumquat (*C. japonica*) they found *in vitro* an inhibitory activity of IC_{50} = 63 µg/mL, a result which is different from ours. This can be explained by the difference in the species studied.

It has been recognized that polyphenols and flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. According to our study, the high contents of these phytochemicals in *Fortunella margarita* can explain its high radical scavenging activity. Kumquats are also an excellent source of nutrients and phytochemicals, including ascorbic acid, carotenoids, flavonoids and essential oils.[35] The radical scavenging activities of all extracts were observed to increase with increasing concentration. Results are in agreement with previous data indicating high antioxidant effect of kumquat.[36] The high phenol and flavonoids contents of peels of *Fortunella margarita* may be responsible of the high antioxidant activity of this plant.

Essential oils are fairly complex mixtures consisting of several tens of components, and this complexity often makes it difficult to interpret patterns of activity. For this reason, many reports on the antioxidant potential of essential oils often refer to the concepts of synergy, antagonism, and additive effects.[37] However, in our study, the essential oil of *Fortunella margarita* peels exhibited higher levels of limonene and monoterpenes, but weaker antioxidant properties, which was attributed to its overall performance as an antioxidant, which is the result of complex interactions between components and oxidizable protected substances. Depending on experimental conditions and essential oil composition, synergistic

or antagonistic behaviour is expected, and there may be exceptions, which may be an area for further research on the essential oil of *Fortunella margarita* peels.[38]

The antioxidant properties of essential oils with oxygenated sesquiterpenes were revealed by Mohammedi and Atik.[39] Similarly, Mimica-Dukic *et al*. [40] revealed the DPPH radical is neutralized by sesquiterpenes (caryophyllyene) and monoterpenes (limonene). On the other hand, essential oil from *Citrus* x *limon* (L.) Burm. f., which has a high concentration of limonene, did not correlate to the best activity.[41] Furthermore, Kelen and Tepe[42] found that the monoterpenes (limonene, α-pinene and β-pinene) tested individually did not have a high antioxidant activity compared to the same constituents when tested together. This suggests a synergy between these compounds.

Toxicity assay was conducted to determine the toxic activity of the essential oil of *Fortunella margarita.* In this study, the essential oil from *Fortunella margarita* showed an LC_{50} value of 70 µg/mL. According to Meyer *et al*.[43] lethal concentrations below 1000 µg/mL indicate the presence of potentially active substances. Based on previous research the essential oil of *Fortunella margarita* has a pharmacological effect and may have promising potential for further development in anticancer drugs.[44]

To the best of our knowledge, this is the first report of the toxic activity by means of *Artemia salina* lethality assay of essential oil of *Fortunella margarita.* These findings suggest that the test may expedite toxicity experiments and decrease costs, and therefore, may be considered an alternative to the *in vitro* cell culture assay.

Studies have shown that medicinal plants due to their active ingredients and medicinal and antioxidant compounds have beneficial effects on human health and have a therapeutic effect on various organs of the body and various diseases.[45]

Conclusions

This study focused on the relationship between the chemical composition and efficacy of the essential oil extracted from the peels of *Fortunella margarita*, as well as its antidiabetic, anti-inflammatory, antioxidant, and toxic properties. The studied kumquat has the potential to be a source of flavonoids and phenolic compounds that are antioxidative, anti-inflammatory, and anti-diabetic. The results demonstrate conclusively that this plant's essential oil can serve as a useful natural alternative to modern medicine in the food preservation and pharmaceutical industries. More research should be conducted to identify new compounds and determine which are responsible for these biological activities, as well as to examine their effects *in vivo*.

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