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Isolation and identification of anticancer flavonoids from Valentia orange peel extract: *In vitro* evaluation

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Abstract

Cancer, one of the leading causes of death, has seen progress in the development of drugs for intervention. However, these drugs often come with severe side effects. As a result, there has been a growing interest in functional foods and natural products for their potential as anti-cancer therapies. One example is orange peels (OPs) and their derived extracts, demonstrating strong anti-cancer properties due to their high flavonoid content. OPs contain numerous bioactive compounds that can be used in drug development. This study aimed to isolate flavonoids from OPs and assess their anti-cancer potential against Hep-G2 cancer cells. High-performance liquid chromatography (HPLC) analysis revealed 19 flavonoid compounds in OPs, with chrysin, kaempferol, and catechin being the major constituents. The alcoholic extract of OPs exhibited antioxidant potential against ABTS and DPPH. Six flavonoids were isolated from citrus peel. These are: Kaempferol-3-O-rutinoside (nicotiflorin), Isorhamnetin-3-O-B-rutinoside, Rutin, Qercetin-3-O-β-glucoside, Isorhamnetin and hesperdin. The isolated compounds exhibited higher levels of cytotoxicity to cancer cells. These findings support the use of OPs as potential anticancer agents.

KEYWORDS: Fruit by-products; Citrus peels; Flavonoids; Liver cancer.

Introduction:

Cancer is one of the most lethal diseases in the world [1]. Plants were traditionally used in the past to treat cancer. Therefore, Medicinal plants are considered a good starting point and an interesting pool for discovering new anticancer drugs [2-7]. Recently, the use of fruit processing by-products has increased. The potency the lack of side-effects and the cost of by-products compared to current therapies to treat dangerous diseases such as cancer and Alzheimer's disease make them more attractive [8-12].

Large amounts of byproducts are generated after processing of citrus fruits which contain valuable phytocompounds. In Egypt, more than metric tons of byproducts are annually produced after the processing of citrus fruits. Researchers and consumers have increased the demand to recover natural value-added compounds from citrus wastes [13]. Many studies have been published related to the processing of citrus waste for the recovery of natural value-added compounds and bioactive compounds like flavonoids [14].

Citrus belongs to the family Rutaceae, and are characterized by their fragrance. It is also well known for its therapeutic uses. The chemical composition of non-edible parts of citrus called citrus waste, such as peel, flavedo, and seeds of fruits that contain seeds. Citrus wastes contain free soluble sugars, starch, fiber, cellulose and hemicelluose, diginin and protein, and many active compounds which must be treated carefully before disposal. The disposal of citrus waste is a major problem. It accounts for 55-60% of fruit weight [15].

Many steps are included for the recovery of the value-added products from citrus waste like, extraction, isolation, purification, and identification of the method depending on the native compounds found in the waste, then these compounds are tested for their bioavailability and later on will be incorporated in or used in food industries, in technological and health-promoting domains. Citrus peels have flavonoids as their anticancer constituents. These bioactive compounds have been found to provide health benefits such as antioxidative, anticancer, and anti-inflammatory protective activities [16]. Herein, our study promoted the use of Ops and their isolated compounds, which are wasted in huge amounts, to ensure its activity in pharmacological effects as anticancer agents.

MATERIAL AND METHODS

Plant material

Peels from mature navel oranges were purchased from the market, and then sliced into small pieces and dried in an oven at 40 degrees Celsius. The dried peel was pulverized and stored in paper pages until used, grinding was necessary to improve extraction efficiency.

Determination of total flavonoid (TF), total phenol (TP) contents, and antioxidant activity of alcoholic OPs extract

The powdered peels were extracted with methanol. The TP content of the alcoholic OPs extract was analyzed using

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the Folin–Ciocalteu reagent using gallic acid (GAE) as standard according to Singleton and Rossi [17]. TF content was estimated using the aluminum chloride method using Catechin (CAT) as standard according to Willet [18]. In addition, free radical scavenging capacity (DPPH and ABTS) was also estimated as described by Hwang and Do Thi [19].

Identification of phenolic and flavonoid compounds

The phenolic and flavonoid compounds of citrus peels were extracted according to the method described by Mattila et al., [20]. HPLC analysis was carried out according to Kim *et al.* [21]. The HPLC system was a HP 1100 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an auto-sampler, quaternary pump, and a diode array detector. The measurements were integrated by Chemstation chromatographic software interfaced with a personal computer. The analytical column was ZORBAX Eclipse XDB C18 column (15 cm x 4.6 mm I.D., 5 µm, USA).

Isolation of hesperidins

Air-dried sweet OPs were grinded into powder and extracted with petroleum ether. The defatted powder was extracted using methanol. The alcoholic extract was concentrated and crystallized by adding acetic acid (6%), yielding orange needles (crude hesperidin) melting point was 268°C. There are two spots were observed in thin layer chromatography (TLC) of crystals using n-Butanol: Acetic Acid: Water (3:1:1) as mobile-phase at 0.20 and 0.62. The white crystalline hesperidin was precipitated using chloroform and then filtered. Pure hesperidin has a melting points 240-253 °C.

Isolation of flavonoids

Air-dried and grounded citrus peels (1500 g) were extracted with 70% ethanol (v/v) at room temperature. After evaporating ethanol under reduced pressure, the residue was dissolved in H_2O and then applied to a polyamide column eluted with 10:80% ethanol. Collected fractions were combined based on their TLC profiles and were concentrated at reduced pressure, after ethanol evaporation of the ethanol-eluted fractions. Each fraction was further purified on a Sephadex LH-20 column using 50% Methanol as the mobile phase to yield six compounds. The structures of the isolated compounds were elucidated by EI-MS (JMS-SX102A spectrometer), C^{13}, and ^{1}H-nuclear magnetic resonance (Bruker BioSpin GmbH, Rheinstetten, Germany) data by comparison with published data.

Anticancer activity

Human liver cancer (Hep-G2) cells were supplied by Naval American Research Unit—Egypt (NAmRU). Cytotoxicity against cancer cells was assessed by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay with slight modification as described by Aboubaker [22].

RESULTS AND DISCUSSIONS

Natural substances like flavonoids have been used in medicine more and more in recent years. Flavonoids are secondary metabolites found in plants that are involved in the synthesis of yellow or reddish-blue pigmentation or in protecting flowers from microbes and insects. They also have potential medical uses due to their pharmacological, antioxidant, and antibacterial properties. Numerous epidemiological studies [23-25] have demonstrated a connection between the consumption of flavonoids and the risk of cardiovascular disease and cancer. High-performance liquid chromatography (HPLC) has been utilized in this work to identify the flavonoids in OPs. The phenolic and flavonoids in the alcoholic extract of OPs were identified by the use of an easy-to-use and effective HPLC fingerprint approach.

Table (1) displays the alcoholic extract of OPs as determined by HPLC. By comparing the UV spectrum and retention duration of each peak with those of reference compounds, a total of 19 phenolic compounds were estimated. Three of these 19 peaks—chrysin (2.75 mg/g), kaempferol (1.62 mg/g), and catechin (1.29 mg/g)—were found to have a high intensity (Table 1). Two flavonoids present in citrus peels (Albedo and flavedo), exhibited a typical distribution pattern across different fruit kinds or portions [26-29]. Grapefruit species are characterized by naringin and low hesperidin levels [30]. The main characteristics of sour citrus are naringin, neohesperidin, and occasionally neoeriocitrin [31].

High levels in hesperidin and narirutin are found in orange peels [32-33]. Lemon peel flavonoids are characterized by the presence of four groups of flavonoids: rutin; hesperidin; luteolin-7-rutinoside; diglucosyl luteolin, diglucosyl apigenin, diglucosyldiosmetine; and diglucosyl chrysoeriol [34].

Table 1: Identification of phenolic and flavonoid Compounds using HPLC

Compounds	Tr (min)	mg/g extract	Compounds	Tr (min)	mg/g extract
Gallic	3.9	0.34	Sinapic	30.156	0.05
Protocatechuic	7.65	ND	p-coumaric	34.4	0.22
p-hydroxybenzoic	11.88	0.47	Rutin	33.98	0.22
Gentisic	11.8	0.44	Apigenin-7-glucoside	37.93	0.84
Cateachin	14.74	1.29	Rosmarinic	38.8	0.48
Chlorogenic	15.95	0.13	Cinnamic	46.12	0.20
Caffeic	16.79	0.06	quercetin	48.82	0.05
Syringic	18.67	0.26	Kaempferol	55.06	1.62
Vanillic	20.55	0.50	Chrysin	59.03	2.75
Ferulic	28.27	0.55			

Radical scavenging activity

OPs' phytocompounds have a strong antioxidant potential that protects against free radicals. Flavonoids are responsible for the antioxidant capacity of OPs [16]. By contributing protons or electrons, OPs help to stabilize free radicals [35-36]. Compared to H radicals, OPs flavonoids are more compatible with the OH scavenging process and have more antioxidant capacity [37]. Using DPPH and ABTS tests, the antioxidant capacity of OPs alcoholic extract was investigated in this work. Table 2 indicates that the test samples' radical scavenging activity was greater for Trolox than for OPs alcoholic extract. Superior antioxidant capacity against DPPH and ABTS radicals (56.05 and 30.31 mgTrolox/g) was demonstrated by OPs alcoholic extract.

The strong antioxidant capacity of OPs alcoholic extract has been demonstrated in numerous research; its activity is dependent on the quantity of flavonoids (20.55±0.8 mg CE/mg) and phenols (25.606±0.5 mg GAE/mg) that are known to have antioxidant qualities [38-39]. El-aal and

Halaweish [40] revealed that the extract of alcoholic OPs had a 59% DPPH scavenging activity.

Additional research has shown that the alcoholic extract from OPs has an impressive antioxidant potential. Specifically, when compared to the volatile fractions, the polar fractions of citrus peels demonstrated the best potential for antioxidant activity [41]. Because of the high concentration of flavonoids and phenolic acid esters in the ethyl acetate subfraction of OPs, it demonstrated significant antioxidant potential [42].

OPs contained a high level of tangeritin and nobiletin and also showed superior antioxidant capacity against DPPH and ABTS [36]. The antioxidant activity of the citrus peel has been reported to be higher than that of the fruit and the pulp [43]. OPs contain the highest levels of vitamin C, and flavonoids reported for different citrus species compared to pulp and juices and this contributes to the potent antioxidant potential of the peels than the pulp [16].

Due to variations in phenolics and flavonoids, citrus peels from different species have varying levels of antioxidant potential [43]. Of the different citrus species, Citrus reticulata's alcoholic extract had the highest antioxidant capacity, while *Citrus aurantium*'s antioxidant capacity was the lowest [44]. *Citrus sinensis* and *Citrus reticulata* had the highest antioxidant activity values, which varied from 11.0 to 46.1 mol TE/g for different citrus fruits flavedo extracts [45]. According to Chen et al. [36], the alcoholic extract of fresh OPs demonstrated IC_{50} values of 1..99 and 2.05 mg/mL in the ABTS and DPPH scavenger methods. Additionally, TP content and anti-radical activity were found to be strongly correlated in the study conducted by Lagha-Benamrouche and Madani [46]. The OPs have a high TP content and show high antioxidant capacity.

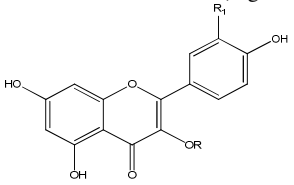
Table 2. TP and TF contents, the antioxidant activity of OPs alcoholic extract

Test	Alcoholic extract of OPs
TP content	25.606±0.5 mg GAE/mg
TF content	20.55±0.8mg CE/mg
DPPH	30.31 mg Trolox/g
ABTS	56.05 mg Trolox/g

Mean of 3 replicates ± st.dev

Identification of isolated compounds

The further chemical characterization of the OPs alcoholic extract via different chromatographic tools as well as spectroscopic techniques led to the isolation and identification of five main flavonoids (Figure 1).



Cpd	R	R_1
F1	**H**	**Rutinoside**
F2	**OMe**	**Rutinoside**
F3	**OH**	**Rutinoside**
F4	**OH**	**Glucopyranoside**
F5	**OMe**	**H**

Figure 1: Isolated compounds from Citrus peels.

Kaempferol-3-O-rutinoside (nicotiflorin) (F1): Was isolated as yellow amorphous powder, ^{1}H-NMR (CD_3OD, 500 MHz): δ: 8.11 (H-2′, 6′, *J* = 9.5 Hz), 6.91 (H-3′, 5′, d, *J* = 9.5 Hz), 6.38 (H-6, d, *J* = 1.6 Hz), 6.18 (H-8, d, *J* = 1.6 Hz), 5.13 (H-1′, d, *J* = 7.1 Hz), 4.49 (H-1″, d, *J* = 1.2 Hz), and 1.09 (CH_3, d, *J* = 6.1 Hz). ^{13}C-NMR (CD_3OD, 125 MHz): δ: 16.6 (C-5‴), 76.5 (C-1″), 75.6 (C-2″), 74.1 (C-3″), 72.3 (C-4″), 71.0 (C-6″), 70.8 (C-1‴), 70.2 (C-2‴), 68.1 (C-3‴), and 67.3 (C-4‴)., EI-MS m/z: 595.4 $[M+H]^+$.

^{13}C-NMR(CD_3OD, 500 MHz): δ:159.4 (C-2), 135.3 (C-3), 179.3(C-4), 162.9(C-5), 99.9 (C-6), 166.2(C-7), 95.2 (C-8), 158.7(C-9), 105.7(C-10), 122.8 (H-1′), 132.5 (H-2′), 116.3 (H-3′), 161.3 (H-4′), 116.7 (H-5′), 132.4 (H-6′), 104.5(H-1″), 75.9 (H-2″), 78.1 (H-3″),71.5 (H-4″), 77.3 (H-5″), 68.7 (H-6″), 102.3 (H-1‴), 72.1 (H-2‴), 72.4 (H-3‴), 73.8 (H-4‴), 69.6 (H-5‴), 17.8 (H-6‴).

Isorhamnetin-3-*O*-B-rutinoside (narcissin) **(F2)**: was isolated as yellow powder^{1}H-NMR(CD_3OD, 500 MHz): δ:6.26 d (*J*=2.0, H-6), 6.46 d (*J*=2.0, H-8), 7.99 d (*J*=2.0, H-2′), 6.96 d (, *J*=8.4, H-5′), 7.67 dd (*J*=8.4, 2.0, H-6′), 3.99 s (OCH_3), 5.29 d (*J*=7.5, H-1″), 3.27–3.55 m (H-2″-H-5″), 3.84 dd (*J*=11.5, 1.6, H-6″-a), 3.41–3.50 m (H-6″-b), 4.53 d (*J*=1.5, H-1‴), 3.62 dd (*J*=3.4, 1.6, , H-2‴), 3.27–3.57 m (H-2‴- H-5‴), 1.16 d (*J*=6.1, H-6‴).^{13}C-NMR (CD_3OD, 500 MHz): δ:159.1 (C-2), 135.6 (C-3), 178.9 (C-4), 162.99 (C-5), 99.8 (C-6), 166.3(C-7), 95.0 (C-8), 158.3 (C-9), 105.4 (C-10), 122.9 (H-1′), 114.7 (H-2′), 148.1 (H-3′), 151.0 (H-4′), 115.9 (H-5′), 124.2 (H-6′), 55.9 (OCH_3), 104.7 (H-1″), 76.1 (H-2″), 77.9 (H-3″), 71.4 (H-4″), 77.1 (H-5″), 68.52 (H-6″), 102.7 (H-1‴), 72.3 (H-2‴), 72.5 (H-3‴), 73.9 (H-4‴), 69.9(H-5‴), 17.88(H-6‴).EI-MS m/z: 625.2 $[M+H]^+$.

Quercetin-3-*O*-*β*-D-rutinoside (**Rutin) (F3):** was isolated as yellow amorphous powder. ^{1}H-NMR (CD_3OD, 500 MHz): δ:6.28(H-6, d, *J*= 2.0Hz), 6.46(H-8, d, *J*=2.0Hz), 7.74(H-2',d, *J*=2.1Hz), 6.91(H-5',d, *J* = 8.5 Hz), 7.66 (H-6', dd, *J* =8.5, 2.1 Hz), 5.16(H-1",d, J=7.5 Hz), 4.53(H-1'",d, J= 1.5 Hz,), δ 1.14(CH_3-rha, d, J= 6.3 Hz) 3.23-3.88(m, the rest of both glucose and rhamnose).EI-MS m/z: 611.1 $[M+H]^+$.

Qercetin-3-*O*-β-glucoside (F4): was isolated as amorphous brown powder. .^{1}H-NMR (CD_3OD, 500 MHz): δ:6.22 (H-6, d, *J*= 2.0 Hz),6.29 (H-8, d, *J*= 2.0 Hz), 7.67 (H-2', d, J:2.0 Hz),7.52 (H-6', dd,J=8.3, 2.1 Hz), 6.90 (H-5', d, J= 8.3Hz,),5.16 (H-1", d, J= 7.6 Hz) 3.19-3.75 (glucose remaining protonds).EI-MS m/z: 464.1$[M+H]^+$.

Isorhamnetin (F5): was isolated as yellow amorphous powder. ^{1}H-NMR (CD_3OD, 500 MHz): δ:3.93(OCH_3, s), 6.31(H-6, d, *J*=2.1), 6.53(H-8, d, *J*=2.1), 7.04 (H-5′, d, *J*=8.4), 7.74(H-6′, m), 7.82(H-2′, d, *J*=2.0).EI-MS m/z: 317.2$[M+H]^+$.

Anticancer activity

Citrus and its bioactive components have been shown to have anticancer properties in several cancer cell lines [47-50]. According to a recent report, eating citrus fruit prevents stomach cancer from developing in the heart [51]. However, only a limited amount of research has been done on OPs' ability to prevent cancer.

While the three isolated compounds' IC_{50} values ranged from 6.96 to 31.72 µg/ml, the various OPs extracts in this study demonstrated inhibition against Hep-G2 cancer cells, with IC_{50} values ranging from 10.79 to 289.17 µg/ml (Table 3). Compared to various OPs extracts, the isolated compounds (kaempferol and isorhamnetin) exhibited higher levels of cytotoxicity to cancer cells, with IC_{50} values ranging from 6.96 to 9.30 µg/ml .

Numerous pieces of evidence suggest that Kaempferol triggers the death of cancer cells by triggering signaling pathways linked to apoptosis. Research has demonstrated that in response to Kaempferol-mediated energy limitation, carcinoma cells initiate AMP-activated protein kinase-dependent autophagy as a survival strategy [52]. Notably, Kaempferol reduces the quantity of different types of cancer cells by means of multiple mechanisms, such as cell cycle arrest, activation of proapoptotic proteins, inhibition of antiapoptotic proteins, and attenuation of prosurvival protein phosphorylation and activity, as well as activation of autophagy or apoptosis [53].

Likewise, Isorhamnetin has come to light for its capacity to inhibit tumor growth in a variety of human malignancies, including colorectal, cutaneous, lung, and breast cancers, by preventing cell migration and proliferation and encouraging apoptosis. The potential of isorhamnetin in the treatment of cancer and the molecular processes underlying apoptosis induction remain unexplored, despite a great deal of study on the cytostatic and pro-apoptotic capabilities of isorhamnetin [54-58]

Many of the bioactive-ingredients present in the OPs extract are antioxidants that provide protection against oxidative stress and this in turn confers additional cancer prevention/protection capacity in the body [59-60].

Table 3: In vitro cytotoxicity of citrus peel fractions against HepG2 cancer cell line determined by MTT assay

	HepG2 % cell inhibition							
Conc (µg/ml)	**MeOH extract**	**Pet extract**	**Chcl3 extract**	**EtoAc extract**	**BuOH extract**	**Kaempferol-3-O-rutinoside**	**Isorhamnetin**	**Hesperdin**
0.39	58.51	38.34	35.62	42.40	46.99	29.71	31.99	39.53
1.56	67.86	50.25	43.40	54.48	51.31	40.44	44.05	53.44
6.25	79.64	59.04	53.93	65.35	60.52	52.04	53.30	63.06
25	93.09	67.61	63.09	75.84	73.57	60.42	62.12	73.60
100	99.70	75.48	72.31	86.09	82.64	71.44	72.81	82.28
IC_{50}	289.17	21.42	10.79	41.41	42.94	6.96	9.30	31.72

It has been suggested that eating citrus prevents heart cancer from developing [51]. Citrus was used as an anticancer agent in antiquity and the Middle Ages, according to Arias and Ramón-Laca [61]. Citrus varieties have also been shown to have anticancer potential on several cancer cell lines. According to the majority of these studies, citrus plants contain flavonoids, which act as antioxidants and lower the risk of cancer [47-50].

Conclusion

The goal of this study was to isolate flavonoids from OPs and evaluate their anti-cancer potential against Hep-G2 cancer cells. High-performance liquid chromatography (HPLC) examination identified 19 flavonoid components in OPs, with chrysin, kaempferol, and catechin being the most abundant. The alcoholic extract of OPs demonstrated antioxidant activity against ABTS and DPPH. Six flavonoids were isolated from citrus peel. These include nicotiflorin (kaempferol-3-O-rutinoside), isorhamnetin-3-O-B-rutinoside, rutin, quercetin-3-O-β-glucoside, isorhamnetin, and hesperdin. The isolated flavonoids had higher levels of cytotoxicity against liver cancer. These data support the use of OPs as anticancer drugs. Relationships among the naturally proportioned flavonoids in OPs and their biological activities are even more complex and unexplored. The mechanisms by which these flavonoids exert optimal therapeutical benefits remain to be further elucidated. So, our results recommended testing the anticancer potential of OPs extract in vivo models.

Declarations

Ethical Approval

All experimental procedures were conducted in accordance with the guide for the care and use of laboratory cell culture procedures were performed in accordance with the Ethics Committee of the National Research Centre with approval certificate registration number 16/138, Experimental procedures and use of laboratory cell culture followed the recommendations of the National Institutes of Health (Publication No. 85-23, revised 1985).

Competing interests

There is no Conflict of interest to declare.

Authors' contributions

Doha H Abou Baker, responsible for isolation of flavonoids, cytotoxicity part, writing and submitting. Elshimy responsible for the identification of the isolated compounds. Emad Hassan the co-PI of the project and responsible for the idea and reviewing. Souad El Gengaihi the PI of the project and responsible for the idea and reviewing.

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Availability of data and materials

Available

References

[1] Holohan, C., Van Schaeybroeck, S., Longley, D.B. and Johnston, P.G., 2013. Cancer drug resistance: an evolving paradigm. Nature Reviews Cancer, 13(10), pp.714-726.

[2] Pérez, A.J., Hassan, E.M., Pecio, Ł., Omer, E.A., Kucinska, M., Murias, M. and Stochmal, A., 2015. Triterpenoid saponins and C-glycosyl flavones from stem bark of Erythrina abyssinica Lam and their cytotoxic effects. *Phytochemistry Letters*, *13*, pp.59-67.

[3] Allam, S.F., Soudy, B.A.N., Hassan, A.S., Ramadan, M.M. and Baker, D.A., 2018. How do mentha plants induce resistance against Tetranychus urticae (Acari: Tetranychidae) in organic farming?. *Journal of Plant Protection Research*, pp.265-275.

[4] El Gengaihi SE, Arafa MM, Abou Baker D.H, Shoaib RM, Asker MS, Abdelhamid SA, Hassan EM (2020). Chemical, Biological and Molecular studies on different Citrus species wastes. Plant archeives, 20(1): 2773-2782.

[5] Abou Baker, D.H., Rady H.M. 2020. Bioassay-guided approach employed to isolate and identify anticancer compounds from *Physalis peruviana* calyces. Plant archives. 20, 3285-3291.

[6] Abou Baker, D.H., Ibrahim, B.M., Hassan, N.S., Yousuf, A.F. and El Gengaihi, S., (2020a). Exploiting *Citrus aurantium* seeds and their secondary metabolites in the management of Alzheimer disease. Toxicology Reports.7: 723-729.

[7] Abou Baker D.H., Moghazy M., AlSayed A.A. (2020b) The in vitro cytotoxicity, antioxidant and antibacterial potential of *Satureja hortensis* L. essential oil cultivated in Egypt. Bioorg. Chem. 103559.

[8] Salam M.A., B.M. Ibrahim, S.E. El-Batran, S.E. El-Gengaihi, D.H. Aboubaker, Study of the possible antihypertensive and hypolipidemic effects of an herbal mixture on l-name-induced hypertensive rats, Asian J. Pharm. Clin. Res. 9(2016) 85-90.

[9] El-Gengaihi S.E., A.T.H. Mossa, A.A. Refaie, D.H. Aboubaker, Hepatoprotective efficacy of *Cichorium intybus* L. extract against carbon tetrachloride-induced liver damage in rats. J. Diet. Suppl. 13 (2016) 570-584.

[10] El-GengaihiS.E., M.A. Hamed, D.H. Aboubaker, A.T. Mossa, Flavonoids from sugar beet leaves as hepatoprotective agent, Int. J. Pharm. Pharm. Sci. 8 (2016) 281-286.

[11] IbrahimE.A., D.H. Aboubaker, and F.K. El-Baz, Anti-Inflammatory and Antioxidant Activities of Rhubarb Roots Extract, Int. J. Pharm. Sci. Rev. Res. 17 (2016) 93-99.

[12] Mossa, A.T.H. F.M. Ibrahim, S.M. Mohafrash, D.H. Aboubaker, S. El Gengaihi, Protective effect of ethanolic extract of grape pomace against the adverse effects of cypermethrin on weanling female rats. Evid. Based Complement. Alternat. Med. (2015), pp. 1-10.

[13] Abou Baker, D.H., Ibrahim, B.M., Abdel-Latif, Y., Hassan, N.S., Hassan, E.M. and El Gengaihi, S., 2022. Biochemical and pharmacological prospects of *Citrus sinensis* peel. Heliyon, 8(8).

[14] Abou Baker, D.H., Ibrahim, E.A. and Salama, Z.A.E.R., 2021. Citrus peels as a source of bioactive compounds with industrial and therapeutic applications. Phenolic Compounds—Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications, pp.1-13.

[15] Kalra, K.L., Grewal, H.S. and Kahlon, S.S., 1989. Bioconversion of kinnow-mandarin waste into single-cell protein. MIRCEN journal of applied microbiology and biotechnology, 5(3), pp.321-326.

[16] de Moraes Barros, H.R., de Castro Ferreira, T.A.P. and Genovese, M.I., 2012. Antioxidant capacity and mineral content of pulp and peel from commercial cultivars of citrus from Brazil. *Food chemistry*, *134*(4), pp.1892-1898.

[17] Singleton V.L., J.A. Rossi, Colorimetry of TPics with phosphomolybdic-phosphotungstic acid reagents, Am. J. Enol. Viticult. 16 (1965) 144-158.

[18] Willett, W.C., 2002. Balancing life-style and genomics research for disease prevention. Science, 296(5568), pp.695-698.

[19] Hwang E.S. , N. Do Thi, Effects of extraction and processing methods on antioxidant compound contents and radical scavenging activities of laver (Porphyra tenera), Prev. Nutr. FoodSci. 19(2014) 40-48.

[20] Mattila, P., Astola, J. and Kumpulainen, J., 2000. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. *Journal of Agricultural and Food Chemistry*, *48*(12), pp.5834-5841.

[21] Kim, N., Park, K.R., Park, I.S. and Park, Y.H., 2006. Application of novel HPLC method to the analysis of regional and seasonal variation of the active compounds in Paeonia lactiflora. Food chemistry, 96(3), pp.496-502.

[22] Abou Baker, D.H., 2020. *Achillea millefolium* L. ethyl acetate fraction induces apoptosis and cell cycle arrest in human cervical cancer (HeLa) cells. Annals of Agricultural Sciences, 65(1), pp.42-48.

[23] Hollman, P.H. and Katan, M.B., 1999. Dietary flavonoids: intake, health effects and bioavailability. *Food and chemical toxicology*, *37*(9-10), pp.937-942.

[24] Beecher, G.R., 2003. Overview of dietary flavonoids: nomenclature, occurrence and intake. *The Journal of nutrition*, *133*(10), pp.3248S-3254S.

[25] Abou Baker, D.H., 2022. An ethnopharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up to date knowledge. Toxicology Reports, 9: 445-469

[26] Ooghe, W. C.; Detavernier, C. M. Detection of the addition of Citrus reticulata and hybrids to Citrus sinensis by flavonoids. J. Agric. Food Chem. 1997, 45, 1633-1637.

[27] Mouly, P. P.; Gaydou, E. M.; Faure, R.; Estienne, J. M. Blood orange juice authentication using cinnamic acid derivates. Variety differentiations associated with flavanone glycoside content. J. Agric. Food Chem. 1997, 45, 373-377 of phenolic compounds; Trends Plant Sci. ReV. 1997, 2, 152-159.

[28] Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. Flavanone glycosides in Brazilian orange juice. Food Chem. 1998, 61, 275- 280.

[29] Leuzzi, U.; Caristi, C.; Panzera, V.; Licandro, G. Flavonoids in pigmented orange juice and second-pressure extracts. J. Agric. Food Chem. 2000, 48, 5501-5506.

[30] Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin and neohesperidin in Citrus. J. Agric. Food Chem. 1987, 35, 1027-1030.

[31] Marini, D.; Balestrieri, F. Multivariate analysis of flavanone glycosides in citrus juices. Ital. J. Food Sci. 1995, 3, 255-264.

[32] Sendra, J. M.; Navarro, J. L.; Izquierdo, L. C18 solid-phase isolation and high-performance liquid chromatography/ultraviolet diode array determination of fully methoxylated flavones in citrus juices. J. Chromatogr. Sci. 1988, 26, 443-448.

[33] Robards, K.; Li, X.; Antolovich, M.; Boyd, S. Characterization of citrus by chromatographic analysis of flavonoids. J. Sci. Food Agric. 1997, 75, 87-101.

[34] Miyake, Y.; Yamamoto, K.; Morimitsu, Y.; Osawa, T. Isolation of C-Glucosylflavone from lemon peel and antioxidative activity of flavonoid compounds in lemon fruit. J. Agric. Food Chem. 1997, 45, 4619-4623.

[35] Chen, X.M. and Kitts, D.D., 2017. Flavonoid composition of orange peel extract ameliorates alcohol-induced tight junction dysfunction in Caco-2 monolayer. *Food and Chemical Toxicology*, *105*, pp.398-406.

[36] Chen, X.M., Tait, A.R. and Kitts, D.D., 2017. Flavonoid composition of orange peel and its association with antioxidant and anti-inflammatory activities. *Food chemistry*, *218*, pp.15-21.

[37] Kanaze, F.I., Termentzi, A., Gabrieli, C., Niopas, I., Georgarakis, M. and Kokkalou, E., 2009. The phytochemical analysis and antioxidant activity assessment of orange peel (Citrus sinensis) cultivated in Greece–Crete indicates a new commercial source of hesperidin. *Biomedical Chromatography*, *23*(3), pp.239-249.

[38] Lee, D.H., Woo, J.K., Heo, W., Huang, W.Y., Kim, Y., Chung, S., Lee, G.H., Park, J.W., Han, B.K., Shin, E.C. and Pan, J.H., 2022. Citrus junos Tanaka Peel Extract and Its Bioactive Naringin Reduce Fine Dust-Induced Respiratory Injury Markers in BALB/c Male Mice. *Nutrients*, *14*(5), p.1101.

[39] Liu, N., Yang, W., Li, X., Zhao, P., Liu, Y., Guo, L., Huang, L. and Gao, W., 2022. Comparison of characterization and antioxidant activity of different citrus peel pectins. *Food Chemistry*, *386*, p.132683.

[40] Abd El-aal, H.A. and Halaweish, F.T., 2010. Food preservative activity of phenolic compounds in orange peel extracts (Citrus sinensis L.). *Lucrări Ştiinţifice-Seria Zootehnie*, *53*(15), pp.233-240.

[41] Guimarães, R., Barros, L., Barreira, J.C., Sousa, M.J., Carvalho, A.M. and Ferreira, I.C., 2010. Targeting excessive free radicals with peels and juices of citrus fruits: grapefruit, lemon, lime and orange. *Food and Chemical Toxicology*, *48*(1), pp.99-106.

[42] Anagnostopoulou, M.A., Kefalas, P., Papageorgiou, V.P., Assimopoulou, A.N. and Boskou, D., 2006. Radical scavenging activity of various extracts and fractions of sweet orange peel (Citrus sinensis). *Food chemistry*, *94*(1), pp.19-25.

[43] Gorinstein, S., Cvikrová, M., Machackova, I., Haruenkit, R., Park, Y.S., Jung, S.T., Yamamoto, K., Ayala, A.L.M., Katrich, E. and Trakhtenberg, S., 2004. Characterization of antioxidant compounds in Jaffa sweeties and white grapefruits. *Food chemistry*, *84*(4), pp.503-510.

[44] Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M.A., 2009. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pak J Pharm Sci*, *22*(3), pp.277-281.

[45] Ramful, D., Bahorun, T., Bourdon, E., Tarnus, E. and Aruoma, O.I., 2010. Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application. *Toxicology*, *278*(1), pp.75-87.

[46] Lagha-Benamrouche, S. and Madani, K., 2013. Phenolic contents and antioxidant activity of orange varieties (Citrus sinensis L. and Citrus aurantium L.) cultivated in Algeria: Peels and leaves. *Industrial Crops and Products*, *50*, pp.723-730.

[47] KunduSen, S., Gupta, M., Mazumder, U.K., Haldar, P.K., Saha, P. and Bala, A., 2011. Antitumor activity of Citrus maxima (Burm.) Merr. leaves in Ehrlich's Ascites Carcinoma cell-treated mice. *International Scholarly Research Notices*, *2011*.

[48] Song, J.K. and Bae, J.M., 2013. Citrus fruit intake and breast cancer risk: a quantitative systematic review. *Journal of Breast Cancer*, *16*(1), pp.72-76.

[49] Navarra, M., Ursino, M.R., Ferlazzo, N., Russo, M., Schumacher, U. and Valentiner, U., 2014. Effect of Citrus bergamia juice on human neuroblastoma cells in vitro and in metastatic xenograft models. *Fitoterapia*, *95*, pp.83-92.

[50] Patil, J.R., Chidambara Murthy, K.N., Jayaprakasha, G.K., Chetti, M.B. and Patil, B.S., 2009. Bioactive compounds from Mexican lime (Citrus aurantifolia) juice induce apoptosis in human pancreatic cells. *Journal of agricultural and food chemistry*, *57*(22), pp.10933-10942.

[51] Bae, J.M. and Kim, E.H., 2016. Dietary intakes of citrus fruit and risk of gastric cancer incidence: an adaptive meta-analysis of cohort studies. *Epidemiology and health*, *38*..

[52] Filomeni G., Desideri E., Cardaci S., Graziani I., Piccirillo S., Rotilio G., Ciriolo M.R. Carcinoma cells activate AMP-activated protein kinase-dependent autophagy as survival response to kaempferol-mediated energetic impairment. Autophagy. 2010;6(2):202–216.

[53] Kashyap, D., Sharma, A., Tuli, H.S., Sak, K., Punia, S. and Mukherjee, T.K., 2017. Kaempferol–A dietary anticancer molecule with multiple mechanisms of action: Recent trends and advancements. Journal of functional foods, 30, pp.203-219.

[54] Luo, W., Liu, Q., Jiang, N., Li, M. and Shi, L., 2019. Isorhamnetin inhibited migration and invasion via suppression of Akt/ERK-mediated epithelial-to-mesenchymal transition (EMT) in A549 human non-small-cell lung cancer cells. Bioscience Reports, 39(9), p.BSR20190159.

[55] Hu, S., Huang, L., Meng, L., Sun, H., Zhang, W. and Xu, Y., 2015. Isorhamnetin inhibits cell proliferation and induces apoptosis in breast cancer via Akt and mitogen-activated protein kinase kinase signaling pathways. Molecular Medicine Reports, 12(5), pp.6745-6751.

[56] Jaramillo, S., Lopez, S., Varela, L.M., Rodriguez-Arcos, R., Jimenez, A., Abia, R., Guillen, R. and Muriana, F.J., 2010. The flavonol isorhamnetin exhibits cytotoxic effects on human colon cancer cells. Journal of agricultural and food chemistry, 58(20), pp.10869-10875.

[57] Du, Y., Jia, C., Liu, Y., Li, Y., Wang, J. and Sun, K., 2021. Isorhamnetin enhances the radiosensitivity of A549 cells through interleukin-13 and the NF-κB signaling pathway. Frontiers in Pharmacology, 11, p.610772.

[58] Ruan, Y., Hu, K. and Chen, H., 2015. Autophagy inhibition enhances isorhamnetin-induced

mitochondria-dependent apoptosis in non-small cell lung cancer cells. Molecular Medicine Reports, 12(4), pp.5796-5806.

[59] Morley, K.L., Ferguson, P.J. and Koropatnick, J., 2007. Tangeretin and nobiletin induce G1 cell cycle arrest but not apoptosis in human breast and colon cancer cells. *Cancer letters*, *251*(1), pp.168-178.

[60] Arul, D. and Subramanian, P., 2013. Naringenin (citrus flavonone) induces growth inhibition, cell cycle arrest and apoptosis in human hepatocellular carcinoma cells. *Pathology & Oncology Research*, *19*(4), pp.763-770.

[61] Arias, B.A. and Ramón-Laca, L., 2005. Pharmacological properties of citrus and their ancient and medieval uses in the Mediterranean region. Journal of Ethnopharmacology, 97(1), pp.89-95