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Fractionation, Identification and Biological activities of Egyptian Citrus Peel Extracts

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Abstract: Citrus processing by-products represent a rich source of natural occurring flavonoids (hesperidin) and polymethoxylated flavones (nobiletin and tangeritin) which have been of intense interest for their pharmacological properties. Little attention has been given to the isolation of these flavonoids in peel and to the possibility of recovering flavonoids from other citrus peel by-products. Orange peels powder were extracted successively with cold and hot water, ethyl alcohol 95% and 50% chloroform and ether. Cold water extract has the most powerful antimicrobial activity against bacteria and yeast strains. This extract was fractionated by using silica gel column and different solvents into four fractions. Fractions I and II showed the highest antimicrobial activity. They were further fractionated on Sephadex LH-20 using water and ethyl alcohol as solvent systems from which fractions IE and IIE exhibited the most powerful antimicrobial activity and the lowest minimum inhibitory concentration (MIC) values against tested bacteria and fungi ranged from 130-275 µg/ml. So these fractions are considered the most bioactive fractions. They have no toxicity towards brine shrimp where the LD_{50} was more than 1mg/ml. also fraction IE exhibited higher antioxidant activity (80%) than IIE. The two fractions were shown to belong to the flavonone class. Molecular weights of each fraction and $^1H_1$-NMR, IR and melting point indicated that Didymin, naringin, hespiridin, and naringinin were present in IE. Tetra-O-methylscutellarin, sinensetin, nobiletin, naringin, hespiridin and tangeritin were present in IIE fraction. Throughout this study many targets have been achieved such as isolation and purification of active compounds which could be used in the field of medicine.

Key words: Orange peels; Antimicrobial activity; Antioxidant activity; flavonoids polymethoxylated flavonoids; medicinal field.

INTRODUCTION

Throughout the ages, plants have been used by humans as a source of food, cosmetics, medicine, clothing and even shelter. Plant products also play an important role in the health care systems of the remaining 20 percent of the population who mainly reside in developed countries (Kumarasamy *et al*., 2002).

Fruits and vegetables had conferred to be capable of delivering health benefits besides fulfilling physiological needs, (Kaur and Kapoor, 2001).

Citrus fruits make up the largest sector of the world's fruit production, with more than 100 million tons produced each season. About 34% of citrus fruits are made into juices; therefore, large amounts of residues are formed every year (Li *et al*., 2006). Citrus peels, which comprise the dominant residue, exhibit potent anti-oxidant, antimicrobial and anti-inflammatory activities (Lin *et al*., 2008, Murakami *et al*., 2000 and Dhanavade et al., 2011), and are considered potential sources of functional components (Schieber *et al*., 2001). Except for ascorbic acid, citrus peels contain more bioactive compounds, such as phenolic acids, flavonoids, limonoids, and fibre than do juices (Bocco *et al*., 1998 and Gorinstein *et al*., 2001).

Among the well-known citrus bioactive compounds, flavonoids, especially the citrus unique polymethoxy flavones and flavanone glycosides, attract considerable attention for their significant biological activities (Tripoli *et al*., 2007 and kumar et al., 2011).

Due to their high flavonoid content, citrus peels could be exploited by both pharmaceutical and food industries. In spite of this, the compounds present in citrus peel are usually processed as by-products or wasted, resulting in environmental pollution. One of the main reasons for this is the absence of effective extraction procedures to obtain the flavonoids from the citrus peels (Ma *et al*., 2008).

MATERIALS AND METHODS

2.1. Plant material:

The fresh local orange fruits were collected during November, December and January in order to obtain the fruits at mature stage of development.

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2.2. Sample Preparation and Extraction Experimental Design:

Oranges were washed with tap water, peeled off sharply in order to collect only the flavedo part of the peel and air dried at room temperature (18–20 °C) and darkness for 12 days.

Dried powders (100 g) of the local orange peels were exhaustively extracted with cold and hot distilled water separately, followed by 95 and 50 % ethyl alcohol, then chloroform and finely with ether. Afterwards, the solvents were evaporated under reduced pressure in a rotary evaporator until the extracts (A, B, C, D, E and F) become completely dry to give six extracted fractions.

2.3. Antimicrobial Activity:

The determination of antimicrobial activity was carried out by the agar diffusion test in solid media based on cup – plate technique that was described by Deans and Svoboda (1990).

2.4. Purification of the Cold Water Extract of Orange Peels:

The dried cold water extract of the local orange peels that has high antimicrobial effect was dissolved in distilled water and fractionated by chromatography on silica gel column. The column was eluted initially with ethanol (95 %) followed by ethanol: water (3:2) then by ethanol: water: acetone (1:1:2) to give four fractions (I, II, III and IV). Ethyl alcohol fraction was divided to fraction I and fraction II according to the presence of tannin.

2.5. Purification of the Selected Silica Gel Fractions:

2.5.1. Purification by Sephadex LH-20 Column:

Subsequent separation of the fractions by re-chromatography over sephadex LH-20 column (2 X 40 cm) was done separately using distilled water followed by absolute ethanol as eluates.

2.5.2. Purification by Polyamide S column:

The resultant most active antimicrobial fractions from sephadex LH-20 column (IE and IIE) were further purified using a preparative polyamide S column (30x2.5 cm).

2.5.3 Purification by Separation of the Compounds by Polyamide TLC Aluminum Sheet:

The isolated fractions (IE and IIE) were further purified and each was separated on polyamide TLC aluminum sheets, using butanol: acetic acid: water (4:1:5) V/V/V as a solvent system. (Harborne, 1994).

2.6. Determination of Minimum Inhibitory Concentration (MIC):

The MIC of the tested fractions was determined against some selected microorganisms (*Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Candida tropicals, Fusarium solani* and *Alternaria solani*) by agar diffusion technique. Serial dilutions of the peels cold water extract and the purified fractions with different concentrations (150 to 400 µg) were placed in cups cut out in inoculated agar. Incubation was done for 24 hs at 30°C. Growth was determined by visual examination. MIC was calculated by plotting log concentration against the diameter of inhibition zone. A straight line was obtained, which if extrapolated to the cup diameter, the corresponding is the MIC. MIC was expressed as the lowest concentration of plant extract that produced a complete suppression of colony growth (Rios *et al*., 1988).

2.7. Acute Lethal Toxicity Test:

Bioactive compounds are almost toxic in high doses. Thus, in vivo lethality in a simple zoological organism might be used as a rapid and simplemonitor during the fractionation of bioactive plant extracts so cytotoxicity test was determined by brine shrimp lethality bioassay according to Mayer *et al*. (1982).

2.8. High- Performance Liquid Chromatography (HPLC):

Determination of naturally occurring flavonoids of the orange peels (*Citrus sinensis*) by HPLC was done according to Nogata *et al*. (1994).

High-performance liquid chromatography (HPLC) coupled with ultraviolet-visible spectrophotometry using a photodiode-array detector was used as a routine method for the simultaneous determination of naturally occurring flavonoids. The separation system consisted of a C_{18} reversed-phase column, a gradient system of 0.01 M phosphoric acid (A) and methanol (B), and a photodiode-array detector. Each of the flavonoids was eluted from the column with a gradient system composed of three periods: (1) 0-55 min, 70-55% (v/v) A in B, (2) 55-95 min, 55-0% A in B, and (3) 95-100 min, isocratic, 100% B, and quantified by spectrophotometric detection at 285 nm. Identifications of specific flavonoids were made by comparing their retention times (t_R) and UV spectra with those of standards.

2.9. Assay for Antioxidant Activity:

The anti-oxidant was carried out by measuring the decolorizing capacity (bleaching) of each fraction against the stable 1,1-diphenyl -2- picryl- hydrazyl radical(DPPH). The color change due to radical scavenging can be measured using spectrophotometer at 517 nm (Deby and Margotteaux, 1970).

2.10. Identification of the Flavonoids:

2.10.1. Identification by Determination of the Molecular Weights:

The molecular weights of the purified flavonoids were determined by chomatography on sephadex G-25 column (Oszmianski and Sapis, 1989). Sephadex G-25 (Fine, Pharmacia) was equilibrated in acetone (analytical reagent grade) - water (1:1) and the swollen gel was packed into glass column (2.5 x 45 cm), bed volume (V_b) 225 ml. The flow rate was maintained at 60 ml / h and samples were collected in 2.2 ml fractions. Absorbance versus volume profiles was constructed by measuring the absorbance of each fraction at 350 nm. The dead volume ($V_\circ$) was determined by dextran blue.

A plot of elution volume of the used standards / column bed volume (V_e / V_b) versus log molecular weight for a number of flavonoids of known molecular weight was used for determination the molecular weight of the purified flavonoids .

2.10.2. Identification by UV Spectral Analysis:

The spectral analysis was accomplished by preparing the fractions in methanol and measured in 1cm path quartz cells with the addition of the diagnostic shift reagent by Shimadzu UV 240 (P/N 204-58000) spectrophotometer

2.10.3. Identification by $^1H_1$ NMR Spectra:

The $^1H_1$ NMR spectra were recorded in deuterated Dimethylsulphoxide (DMSO-d6 at 300 MHz on a Varian Mercury VX-300 NMR spectrometer). The data were recorded as δ ppm relative to TMS. Chemical shifts were related to that of the solvent.

2.10.4. Identification by IR Spectra:

IR spectra were recorded on Perken Elmer 457 infrared spectrophotometer using KBr discs.

2.10.5. Identification by Melting Point:

Melting point was determined on Reichest meltry point microscope and they were uncorrected.

RESULT AND DISCUSSION

3.1. Antimicrobial Activity of the Orange Peels Extracts (A, B, C and D):

Table (1) showed the antimicrobial activity of the different extracts (A, B, C and D) of orange peels expressed by the diameter of zone inhibition (mm), and with concentration of 0.5 mg/ml in each well. Cold water extract showed a high antimicrobial activity towards bacteria especially *Bacillus subtilis, Esherichia coli*, *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Proteus mirabilis* and yeast especially *Candida albicans* and *Candida tropicals*.

Table 1: Antimicrobial activity of the different extracts of orange peels (zone inhibition method mm).

Microorganisms	Zone Inhibition diameter (mm) at 24h			
	A	B	C	D
Bacillus subtilis	12	9	8	5
Escherichia coli	11	8	7	7
Pesudomonas aeruginosa	13	8	7	6
Salmonella typhi	10	9	7	5
Staphylococcus aureus	12	8	6	5
Proteus mirabilis	9	7	6	5
Candida albicans	12	10	9	8
Candida tropicals	12	9	8	5
Saccharomyces cervisae	10	6	8	5

A : Cold water B : Hot water C : Ethyl alcohol 95 % D : Ethyl alcohol 50%

3.2. Antimicrobial Activity of the Silica Gel Cold Water Extract Fractions (I, II, III and IV) After 24 and 48 hs:

Fractions I and II showed the highest antimicrobial activity against *Candida albicans* and *Candida tropicals* followed by *Bacillus subtilis*, *Escherichia coli*, *Pesudomonas aeruginosa* and *Proteus mirabilis*, also a moderate activity against fungi, *Alternaria solani* and *Fusarium solani* was noticed. After 48 hours of growth

the degree of inhibition was decreased by 30-50% in all tested fractions against examined strains of bacteria, yeast and fungi.

3.3. Purification of the Selected Silica Gel Fractions by Sephadex LH-20:

Fractions I, II and III were further fractionated by Sephadex LH-20 column, (40 x 2 cm). For each fraction two mobile phases were used. The first one used was water followed by absolute ethyl alcohol. 5 ml of eluents were collected in 50 tubes. A peak of fraction I eluted with water, which represent tubes from 10 – 30 (105 ml) and named IW. Most IW fraction contains carbohydrates and proteins. Fig (1) represents the peak of fraction I eluted with ethyl alcohol which represents tubes from 19-30 (60 ml) and named IE. The peak of fraction II eluted with water which represent tubes from 9 – 27 (95 ml) and named IIW which contain carbohydrate and protein. Fig (2), represent the peak of fraction II eluted with ethyl alcohol which represent tubes from 5-40 (180 ml) and named IIE.

A peak of fraction III eluted with water represent tubes from 10–40 (115 ml) and named IIIW. Fig (3) represent a peak of fraction III eluted with ethyl alcohol, which represent tubes from 11-30 (100 ml) and named IIIE.

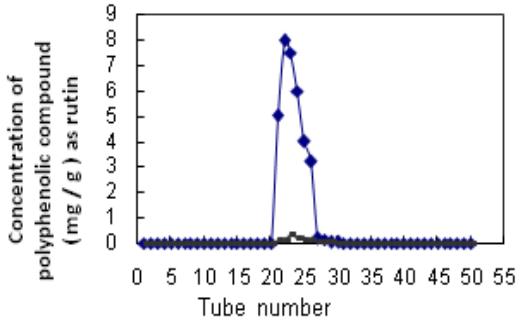
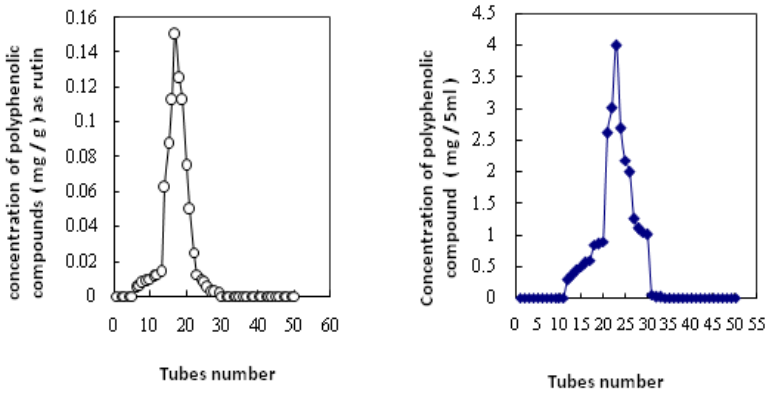


Fig. 1: Elution profile of fraction I on Sephadex LH-20 column using ethyl alcohol as eluent.



Figs. 2,3: Elution profile of fractions II and III on Sephadex LH-20 column using ethyl alcohol as eluent.

Antimicrobial activity of the Sephadex LH-20 fractions was expressed by the diameter of inhibition zone in mm after 24 and 48 hs incubation time. All fractions have different antimicrobial activities toward selected microorganisms. Fractions IE and IIE showed very strong antimicrobial activity on *Candida albicans* and *Candida tropicals.* These fractions showed high antimicrobial activity on *Escherichia coli, Pseudomonas aeruginosa* followed by *Staphylococcus aureus* and *Proteus mirabilis.* Data showed also a moderate antimicrobial activity towered *Fusarium solani* and *Alternaria solani* and a weak activity against *Aspergillus candidus.*

3.4. Determination of Minimum Inhibitory Concentration (MIC):

Table (2) represent the minimum inhibitory concentration of cold water extract, silica gel fractions (I –IV) and Sphadex LH-20 fractions (IE and IIE). All assayed Gram negative bacteria and yeast (*Esherichia coli*, *Pesudomonas aeruginosa*, *Candida tropicals*, *Candida albicans*) were most sensitive to Sephadex LH-20 fractions (IE and IIE) with MIC values of (130 – 275 µg /ml). Fractions IE and IIE showed the lowest MIC

values which were 250 and 265 µg /ml for *Fusarium solani* and 250 and 275 µg /ml for *Alternaria solani*, respectively.

Table 2: Minimum inhibitory concentration of cold water extract, silica gel fractions (I-IV) , Sephadex LH-20 fractions (IE and IIE) of orange peels powder.

	Minimum inhibitory concentration (mg/ml)					
Selected strains	*Esherichia Coli*	*Pesudomonas aeruginosa*	*Candida tropicals*	*Candida albicans*	*Fusarium solani*	*Alternaria solani*
Cold water extract	215	215	200	200	350	350
Fraction I	195	195	180	175	300	295
Fraction II	200	195	185	180	310	315
Fraction III	210	210	215	195	325	330
Fraction IV	250	250	275	265	375	380
Fraction IE	150	160	145	130	250	250
Fraction II E	165	175	160	150	265	275

3.5. Brine Shrimp Lethality Test:

The mortalities of brine shrimp larvae for different doses of orange peels extracts (A, B, C and D) and silica gel fractions (I, II and III) were compared with both negative and positive controls. The negative control gave no mortalities after 24 h. It was found that there are no mortalities at 10, 100 and 1000 µg / ml of the studied orange peels extracts (A, B, C and D) and silica gel fractions (fraction I, II and III). So the LD_{50} of each extract was found to be greater than 1000 µg / ml for the four extracts and the three fractions using the brine shrimp larvae, *Artemia solani*. Thus, the extracts and the fractions of orange peels were relatively non-toxic.

3.6. Identification and Quantitative Determination of Flavonoids in Orange Peels Powder:

Twenty micro litters of the prepared sample of orange peels extract was subjected, side by side, with authentic samples of different flavonoids to HPLC technique.

Identification of the compounds were carried out by running them with authentic samples, comparing the eluted peaks for both, the same typical retention times and the same typical UV absorption pattern.

The concentrations were calculated depending on the absorption (mAu) of each peak area. The concentrations of the obtained flavonoids as well as the retention time are presented in Table (3).

3.7. Antioxidant Activity:

The DPPH free radical scavenging activity of the seven fractions was investigated.The radical scavenging activity of seven fractions (I, II, III, IV, IE, IIE and IIIE) were 75, 70, 50, 45, 80, 75 and 40 %, respectively at (10µg /ml) concentration. Results showed high antioxidant activities of all examined fractions, and as moving gradually from fractions to highly purified fractions (IE and IIE), the antioxidant activity was increased.

Table 3: The retention time and UV maximum of the investigated flavonoids.

No	Common name	Retention time	λ max (nm)	Concentration (mg / 100 g)
1	Eriocitrin	18.1	285	0.06
2	Vitexin	21	285	0.081
3	Narirutin	26	282	0.1
4	naringin	29	284	45
5	Hesperidin	35	285	65
6	didymin.	38	282	0.05
7	Diosmin	44	253, 286,345	0.06
8	Naringenin	55	289	15
9	Diosmetin	70	252,268,347	0.07
10	Tetra-O-methylscutellarein	75	266,318	2
11	Sinensetin	79	240,265,326	0.8
12	Tangeretin	85	271,322	2.25
13	Nobilitin	88	248,272,332	1.25

3.8. Chemical Constituents of IE and IIE Fractions by Polyamide TLC Sheet:

As a result of applying of IE and IIE fractions on polyamide sheet and by using butanol: acetic acid : water (4:1:5) as solvent system, 4 and 6 bands were detected respectively.

Each band was eluted by methanol and then further purified by using Sephadex LH-20 column (20 x 1 cm) with methanol 80% as a mobile phase. The bands were followed along the column by UV lamp to collect each purified compound.

3.9. Identification of the Isolated Flavonoids of IE and IIE Fractions:

By calculating the elution volume/ column bed volume (V_e/ V_b) of each isolated compound of IE and IIE fractions, the molecular weight of each compound was determined which is important in the identification of this compound, Table (4a and 4b).

Table 4a: Compounds of IE fraction.

No of the compound	V_e	V_e / V_b	Mol.wt
1	128	0.57	594
2	131	0.58	580
3	121	0.54	610
4	225	1	272

Table 4b: Compounds of IIE fraction:

No of the compound	V_e	V_e / V_b	Mol.wt
1	200	0.89	342
2	187	0.83	372
3	177	0.787	402
4	131	0.58	580
5	121	0.54	610
6	186	0.826	374

*Note bed volume of the used column ≅ 225 ml.

Chemical analysis which include studying the UV spectrum, $^1H_1$ NMR, IR and determination of the melting point indicated that the four compounds of IE fraction are didymin, naringin, hesperidin and naringenin and the six compounds of IIE fraction are tetra-O-methylscutellarein, sinensetin, nobiletin, naringin, hesperidin and tangeretin.

Epidemiological studies have shown an inverse relationship between dietary flavonoid intake and cardiovascular diseases (Hertog, *et al.*, 1993).

Manthey and Guthrie (2002) have also shown that polymethoxy flavones exhibited a higher anti-proliferative activity than had flavanone glycosides and could serve as potent anti-cancer agents. Indeed, the fact that the hydrophobic nature of the polymethoxy flavones enables them to cross the intestinal membrane easily, and makes them absorbed more readily into the human body (Li, *et al*., 2006). These results indicate that polymethoxy flavones are promising therapeutic phytochemicals of citrus by-products.

Huang and Ho (2010) results suggested that polymethoxy flavones exhibit a greater anti-inflammatory capacity than do the flavanone glycosides. Furthermore, these results support that polymethoxy flavones were the key determinants of the anti-inflammatory activity of citrus peels.

Conclusion:

In this work, a clean, environmental friendly, cheap, available source and efficient methodology which allows extraction of flavonoid from *Citrus sinensis* peels was developed. The strong antimicrobial activity of orange peels isolated fractions on different bacteria and yeast strains beside the antioxidant activity makes orange peels preferable for dietary protection of cardiovascular and other diseases.

REFERENCES

Bocco, A., M.E. Cuvelier, H. Richard and C. Berset, 1998. Antioxidant activity and phenolic composition of citrus peel and seed extract. Journal of Agricultural and Food Chemistry, 46: 2123-2129.

Deans, S.G. and K.P. Svoboda, 1990. The antimicrobial properties of marjoram (*Organum majorna*) volatile oil. *Flavour and Fragrance. J.,* 5: 187-190.

Deby, C. and G. Margotteaux, 1970. "Relationship between essential fatty acids and tissue antioxidant levels in mice''. *C.R.Soc. Biol.,* 164: 2675-2681.

Dhanavade, M.J., Chidamber B. Jalkute, Jai S. Ghosh and Kailash D. Sonawane, 2011. Study Antimicrobial Activity of Lemon (*Citrus lemon* L.) Peel Extract. British Journal of Pharmacology and Toxicology, 2: 119-122.

Gorinstein, S., O. Martin-Belloso, Y.S. Park, R. Haruenkit, A. Lojek and M. Ciz, 2001. Comparison of some biochemical characteristics of different citrus fruits. *Food Chemistry*, 74: 309-315.

Harborne, J.B., 1994. The Flavonoids. Advances in Research Science (1986). Chapman & Hall, London, pp: 261.

Hertog, M.G., P.C.H. Hollman, M.B. Katan and D. Kromhout, 1993. Dietary antioxidant flavonoids and risk of coronary heart disease. *Lancet*, 342: 1007-1011.

Huang, Yung-Sheng and Ho, Su-Chen, 2010. Polymethoxy flavones are responsible for the anti-inflammatory activity. *Food Chemistry,* 119: 868-873.

Kaur, C. and H.C. Kapoor, 2001. Antioxidants in fruits and vegetables- the millennium's health. *International Journal of Food Science and Technology*, 36: 703-725.

Kumar, I.K., M. Narayani, A. Subanthini and M. Jayakumar, 2011. Antimicrobial Activity and Photochemical Analysis of Citrus Fruit Peels-Utilization of Fruit Waste. International *Journal of Engineering Science and Technology*, 3: 5414-5421.

Kumarasamy, Y., P.J. Cox, M. Jaspars, L. Nahar and S.D. Dey, 2002. Screening seeds of Scottish plants for antibacterial activity. *Journal of Ethnopharmacol.,* 83: 73-77.

Li, S., C.Y. Lo and C.T. Ho, 2006. Hydroxylated polymethoxyflavones and methylated flavonoids in sweet orange (*Citrus sinensis*) peel. *Journal of Agricultural and Food Chemistry*, 54: 4176-4185.

Lin, C.C., P.F. Hung and S.C. Ho, 2008. Heat treatment enhances the NO suppressing and peroxynitrite-intercepting activities of kumquat's (*Fortunella margarita* Swingle) peel. *Food Chemistry*, 109: 95-103.

Ma, Y., X. Ye, Y. Hao, G. Xu and D. Liu, 2008. Ultrasound-assisted extraction of hesperidin from Penggan (*citrus reticulate*) peel. *Ultrasonic Sonochemistry*, 15: 227-232.

Manthey, J.A. and N. Guthrie, 2002. Antiproliferative activities of citrus flavonoids against six human cancer cell lines. *Journal of Agricultural and Food Chemistry*, 50: 5837-5843.

Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nicolas and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta medica*, 45: 31-34.

Murakami, A., Y. Nakamura, Y. Ohto, M. Yano, T. Koshiba and K. Koshimizu, 2000. Suppressive effects of citrus fruits on free radical generation and nobiletin, an anti-inflammatory polymethoxyflavonoid. *Biofactor*s, 12: 187-192.

Nogata, Y., H. Ohta, K.I. Yoza, M. Berhow and S. Hasegawa, 1994. High-performance liquid chromatographic determination of naturally occurring flavonoids in *Citrus* with a photodiode- array detector. *Journal of Chromatography,* 667: 59-66.

Oszmianski, J. and J.C. Sapis, 1989. Fractionation and identification of some low molecular weight grape seed phenolics. *Journal of Agricultural and Food Chemistry,* 37: 1293-1297.

Rios, J.L., M.C. Recio and A. Vilar, 1988. Screening methods for natural products with antimicrobial activity: A review of literature. *Journal of Ethnopharmacol*ogy, 23: 127-149.

Schieber, A., F.C. Stintzing and R. Carle, 2001. By-products of plant food processing as a source of functional compounds – recent developments. *Trends in Food Science and Technology*, 12: 401-413.

Tripoli, E., M.L. Guardia, S. Giammanco, D.D. Majo and M. Giammanco, 2007. Citrus flavonoids: Molecular structure, biological activity and nutritional properties: A review. *Food Chemistry*, 104: 466-479.