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The functional food properties of sour orange peel (Citrusaurantium) and its effect on counteract obesity in rats

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Abstract

The aim of this study was to determine the basic components of the Egyptian unripe and ripe sour orange peel, identification of its phenolic and flavonoids compounds and to study the effect of supplementation with two levels from dried unripe (DURSOP) or dried ripe sour orange peel (DRSOP) on feed intake (FI), body weight gain % (BWG %) in male albino rats (SpragoDawley strain), 25 days of age. A total of 36 rats weighting (40±5g) were used. Rats were divided into two main groups, the first main group (n=6) fed on basal diet (BD) and used as a negative control group (-ve). The second main group (30) rats fed on high fat diet all over the experimental period, and then rats were divided into five group as follows: one of them (6 rats) was fed on (HFD) and used as positive control group

(+ve). The other (four subgroups) were fed of (HFD) containing two levels (1.5 or 3%) from (DURSOP or DRSOP). Analysis of the basic components of Egyptian sour orange unripe and ripe peels revealed that protein, fat, ash, fibers, moisture and vitamin C levels were 8.283%,5.911%, 4.968%, 11.63%, 6.26% and 486.82PPM vs. 10.597%, 4.072%, 3.976%, 9.88%, 5.70% and 202.66 PPM, respectively), while the flavonoids compoundsextracted from (DURSOP or DRSOP) revealed the presence of 18 fraction, characterizes with high amount of Naringin, Hespirdin, apig-6rhamnose 8-glucose, Rutin and Quercetrin to be the predominant compound, concerning phenolic compounds results revealed that (DURSOP and DRSOP) resulted in 19 fraction while (DRSOP) showed 21 fraction. The predominant phenolic in (DRSOP) was pyrogallol, which amounted in 9456.49 mg/100gm vs. 354.46 mg/100gm in (DURSOP), results showed that the predominant phenolic in (DRSOP), Isoferulic , Benzoic , Ferulic, Catechein, P-OH-benzoic, caffeine and 3.4,5- methoxy -cinamic. Biological results showed that the supplementation of (HFD) with (DURSOP or DRSOP) at levels (3 or 1.5%), induced a significant reduction in body weight gain %, organs weight / body weight % and peritoneal fat pad %.In conclusion Egyptian sour orange peel considered as potential of natural source of polyphenols and flavonoids compounds that could be assist in management of obesity.

Introduction

Obesity has become the main public health problem in recent decade, because it could increase the risk of chronic disease, such as type two diabetes and coronary heart disease ***(Haslam and Jemes, 2005)***. It is a complex metabolic disorders induced by imbalance between calories intake and metabolic expenditure, which expressed as an increase in a adipocyte number (hyperplasia) and size (hypertrophy) ***(Arner and Spalding, 2010).***

Obesity therapies include reduction of nutrient absorption and administration of drugs that affect lipid mobilization and utilization. Owing to the adverse side effects associated with many anti-obesity drugs, more recent drug trials have focused on screening for natural sources that have been reported to reduce body weight and that generally have minimal side effects ***(Kishino et al., 2006).***

Kang et al., (2012) reported that the peel of citrus SunkiHortextanake which widely used in traditional Asian medin for treatment of many disease, including indigestion and bronchial asthma. Moreover, it significantly decreased the accumulation of fatty droplets in liver tissue and had an anti-obesity effect via elevated B-oxidation and lipolysis in adipose tissue.

Therefore, the aim of this study was to determine the basic components of the Egyptian unripe and ripe sour orange peel and to study the effect of supplementation with two levels from (DURSOP) or (DRSOP) on some nutritional parameters in male albino rats.

Materials and Methods

Materials:

Sour orange (Citrus aurantium) were obtained from El-Oboor market. The raw orange ripe and unripe were washed carefully, and peel cut into small pieces to be exposed to solar energy at National Research Center and then ground to fine power.

Chemical analysis:

Moisture, protein, fat , ash and crudfiber content in sour orange peel (citrus aurantium), were determined according to the method outlined in ***A.O.A.C.(2007),*** vitamin C content determined according to ***(Rodriguez et al., 1992),*** flavonoids were determined according to the method of ***Price et al.,(1978),*** phenolic compounds were determined by HPLC method with UV detector at wavelength 280 nm, according to ***Goupy et al., (1999),*** identification of individual phenolic compounds of samples were performed by HPLC method with UV detector at wavelength 330 nm according to ***(Crozier et al., 1997).***

Chemicals

Vitamins, minerals, casein, cholinechloride and cellulose were purchased from El-Nasr pharm and chemi.Ind comp. Cairo, Egypt.

Rats

Thirty-six male albino rats (SpraguDawely Strain) 25 days of age, weighing (40±5g) were obtained from the laboratory of animal's colony, ministry of healthy and population. Helwan, Cairo, Egypt.

Experimental animals design:

Rats were housed in individual cages under hygienic laboratory condition and were feed on basal diet adlibitum for one week for adaptation in the animal house of faculty of home economics, HelwanUniversity. The basal diet (BD) in the preliminary experiment consists of 14% casein (Protein >85%), soy oil (4%), cellulose (5%), vitamin mixtures (1%), Salt mixtures (3.5%), choline chloride (0.25%) and corn starch (72.25%) ***Reeves et al., (1993).*** The salt mixture and vitamin mixture were prepared according to ***(Hegsted, 1941 and Campbell, 1963).***

After a period of adaptation on BD, rats were divided into two main groups. The first main group (6 rats) fed on BD and was considered (negative control group). The second main group: Thirty rats were fed on high fat diet (HFD) all over the experimental period containing (14 % protein from casein, 20% fat "19% saturated fat : 1% unsaturated fat" , 5% cellulose, 3.5% salt mixture, 1% vitamin mixture , 10% sucrose, 0.25%choline chloride and the remainder is corn starch. Supplementation of diet with dried unripe or ripe dried peel of sour orange was at the experience of starch. Rats of second main group were divided into five subgroups. One of them (6 rats) was fed on (HFD) used as a positive control group and the other four

groups were fed on HFD containing 1.5% dried unripe sour orange peel (DURSOP), 3% (DURSOP), 1.5% dried ripe sour orange peel (DRSOP) and 3% (DRSOP), respectively. During the experimental period (8 weeks) body weight and food consumption were measured twice a week and total food intake of the experimental period was calculated, biological evaluation for different groups ,body weight gain%, body weight% were determined according to ***Chapman et al., (1959) .***

Statistical analysis was carried out using SPSS statistical software version II ***(SAS., 2004)***

Results and discussion

Chemical composition of unripe and ripe sour orange peel:

Results reveled that, the protein, fat, ash, fiber, moisture and vitamin C content were 8.283%, 5.911%, 4.968%, 11.63%,6.26% and 486.82 PPMvs. 10.597%,4.072%, 3.976%, 9.88%, 5.70% and 202.66 PPM, respectively. Our Results revealed that, unripe sour orange peel characterized with high amounts of vitamin C, fat , ash, fiber and moisture than ripe peel while ripe sour orange peel revealed a high content in protein than unripe (10.597 vs. 8.283, respectively) our results are in a agreement with ***(Verpeut et al., 2013).***

Results revealed that vitamin C in unripe and ripe sour orange peel was 486.82 vs. 202.66 PPM, respectively. Results revealed that unripe peel contained vitamin C 2.40 times more than those of ripe peels. Our results are in agreement with ***Diaz et al., (2009).***

Identification of flavonoids compounds of dried unripe and ripe sour orange peels

Table (2) shows the identified flavonoid compounds extracted from unripe and ripe sour orange peels, which fractionated by using high performance liquid chromatography. Our results revealed that unripe and ripe sour orange peels contains 18 fractions. Unripe peel characterizes with high amounts of Naringin, Hespirdin, A pig-6 rhamnose, 8-glucose, rutin and quercetrin , in the amounts of 1774.18, 1568.98, 243.63, 174.70 and 155.61 mg/100g of sample, while the compounds found in the amounts less than 100mg/100g of sample amounted in 88.32, 85.20, 15.82, 22.37, 18.62, 45.14, 48.51, 42.12, 29.10, 45.86, 14.54, 10.39 and 6.08 mg/100g namely A pig-6 arbinose 8-glactose, A pig-7-o-neohespiroside, kamp. 3.7–dirhomoside, Apigenin-7-glucose, Acacetin 7-neo.hesperside, Kaempferol 13-(2-p-camaroyl) glucose, Acacetin neo. hesperside, Quercetin, naringenin, Hespirtin , kampferol, Rhamnetin and Apegnin, respectively). Concerning ripe sour orange peel 18 flavonoids were fractionated from dried ripe peel, namelyhespirdin, naringin and Apig-6- rhamnose 8-glucose showed to be the predominant components which amounted in 1198.59, 400.00 and 237.49 mg/100g, respectively).

Apig.6-arbinose-8- glucose and rutin showed to be less than 100mg/100g sample, which amounted in (92.85 and 51.88mg, respectively) while the major compounds less than 50mg/100g sample showed amounts (26.49, 8.25, 26.96, 4.93, 13.11, 27.37, 4.05, 17.39, 9.78, 9.74 and 1.36mg/100g sample for rutin, Apig-7-o-neohespiroside, kamp-3.7-dirhomoside, quercetrin, A pigenin, 7-glucose,Acecetin, 7-neo-hesperside, Acacetin neo-hesperside, quercetin, narngenin, hespirtin, kampferol, rhamentin and apegnin, respectively),from the above mentioned data it is clear that flavonoid contents in unripe sour orange peel was higher than ripe sour orange peel (1774.18, 1568.98, 243.63 and 174.70 vs. 400.00, 1198.59, 237,49 and 43.30, respectively) for naringin, hesperidin, Apig 6-rhamnose, 8-glocse and rutin, respectively. Our results are in harmony with ***Nakajima et al., (2014).***

In this concern ***Benaventaet al., 1997*** also ***Sun et al., (2013)*** reported that among the flavonoids, citrus present considerable amounts of flavanones , flavones, flavonols and anthocyanins, the main flavonoids are flavanones. In this class of compounds, the most frequent ones are hesperidin andnaringin.

Identification of phenolic compounds of dried unripe and ripe sour orange peel

Table (3) shows the phenolic compounds of dried unripe (DURSOP) and ripe sour orange peel (DRSOP) resulted in 19 fractions, meanwhile (DURSOP) showed that the predominant

phenolic were Pyrogallol , ISO-ferulic, Benzoic , Ferulic , Catechein, P-OH-benzoic, Caffeine and 3,4,5- Methoxy-cinamic which amounted in (3541.46, 435.39, 213.49, 174.39, 143.70, 104.27, 103.22 and 111.60 mg/100 g respectively).

On the other side results revealed that the ripe sour orange peel (DRSOP) showed that the predominant phenolics were Pyrogallol, ISO-ferulic ,Salycilic, P-OH-benzoic , Benzoic , Catechein , Ellagic , caffeine, ferulic, Protocatchuic, Catechol, which amounted in (9456.49, 239.41, 120.44, 84.62, 80.89, 68.72, 61.75, 58.45, 49.49, 48.9 and 48.67 mg/100 g sample, respectively). Results revealed that other phenolic compounds include Coumarin and Alpha coumaric fractions amounted 24.81 and 5.90 mg/100g sample found in (DRSOP). While disappeared in (DURSOP).

Our results revealed that the major phenolic content of the unripe and ripe (DSOP) was composed of pyrogallol(3541.46 vs 9456.49 mg/100g), Catechein (143.70 VS 68.72), P-OH-benzoic (104.27 VS 84.63), Caffeine (103.22 VS 58.45), vanillic (31.33 VS 18.14), while Chlorogenic , Catechol and Proticathuic recorded (21.23 vs. 20.42, 21.18 vs. 48.67 and 21.11 vs. 48.97 respectively).
In this concern ***Kamran et al., (2009)*** reported that phenols and polyphenolic compounds, such as flavonoids have been shown to possess significant antioxidant activities.

Kang et al., (2012) suggested that the peel of citrus sunkihorthadanantiobesity effect via elevated B-oxidation and

lipolysis in adipose tissue. In this respect ***Lu et al., (2013)*** reported that flavonoids are aromatic secondary plant metabolites that are important because of their nutraceuticalvalue, they show several bioactivities such as anti-adipogenic, antiviral antimicrobial and anti-inflammatory activities.

Effect of supplementation with dried unripe or ripe sour orange peel on feed intake, body weight gain % orangs weight / body weight and peritoneal fat pad % of 25 days of age albino rats:

Table (4) illustrate the effect of high fat diet (HFD) supplemented with dried unripe or ripe sour orange peel (DURSOP) or (RSOP) at levels (1.5 or3 %) of feed intake (FI), body weight percent (BWG%), results revealed that all groups 25 days age albino rats which fed on (HFD) for 8 weeks, no abnormal clinical signs were observed during the experimental period.Concerning (FI) results revealed that there is no significant difference ($P<0.05$) between the control negative group, positive control group and the treated groups which fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP) or (DRSOP).

Results revealed that (FI) of albino rats 25 days of age (-ve) group fed on (BD) recorded non-significant difference, as compared with the (+ve) control group fed on (HFD) . All groups fed on (HFD) supplemented with (1.5 or 3%) from (DURSOP or DRSOP) recorded non-significant difference, as compared to the control (-ve) group fed on (BD) Concerning body weight gain%, organs weight / body weight

and peritoneal fat pad %. Results presented in table (4) revealed the effect of (HFD) supplemented with 1.5 or 3 % from (DURSOP or DRSOP) on body weight gain%, organs weight / body weight and peritoneal fat pad %, results revealed a significant increase in BWG %, organs weight /body weight% and peritoneal fat pad of control Positive group, as compared to the negative control group. However the caloric intake of negative group (-ve) fed on (BD) was lower, as compared to the positive group (+ve) fed on (HFD). Our results revealed that there is a significant decrease ($P<0.05$) in (BWG%, organs weight / body weight% and peritoneal fat pad %) of all groups fed on (HFD) supplemented with 3 or 1.5 % from (DURSOP or DRSOP), as compared to the (+ve) control group fed on (HFD).

Except of group feed on HFD supplemented with 1.5% of DURSOP for liver and kidney. we found that there was a non-significant difference between the effect of (DURSOP) and (DRSOP) at level 1.5% on suppressed body weight gain % .

The best results of (DURSOP) or (DRSOP) as anti-obesity effect recorded by groups, which fed on (HFD), supplemented with dried (DURSOP or DRSOP) at 3% followed by 1.5 %.

Our results agreed with the results found by ***Nakajima et al., (2014)*** who reported that citrus polyphenols could assist in the management of obesity, since they cause a reduction in a dipocytedifferentiation, lipid content in the cell and adipocyte apoptosis.

In conclusion, sour orange peel is an important source for bioactive flavonoids, may be a potential natural source for new anti-obesity candidate.

Table (1). Major chemical composition of unripe and ripe sour orange peel are presented in

Samples / source oranges	Chemical composition %					Vitamin C PPM
	Protein	Fat	Ash	Fibers	Moisture	
Unripe peels	8.283	5.911	4.968	11.63	6.26	486.82
Ripe peels	10.597	4.072	3.976	9.88	5.70	202.66

Table (2): Identification of flavonoids Compounds of dried unripe and ripe sour orange peels:

Sample / Flavonoids	Unripe peels mg/100gm	Ripe peels mg/100gm
Apig.6-arbinose 8-glactose	88.32	92.85
Apig.6-rhamnose 8-glucose	243.63	237.49
Naringin	1774.18	400.00
Hesperidin	1568.98	1198.59
Rutin	174.70	43.30
Apig.7-0-neohespiroside	85.20	26.49
Kamp.3.7-dirhamoside	15.82	8.25
Quercetrin	155.61	26.96
Apigenin-7-glucose	22.37	4.93
Acecetin-7-neo hesperside	18.62	13.11
Kaempferol13-(2-p-comaroyl)glucose	45.14	51.88
Acacetin neo. rusperside	48.51	27.37
Quercetin	42.12	4.05
Narngenin	29.10	17.39
Hespirtin	45.86	39.96
Kampferol	14.54	9.78
Rhamentin	10.39	9.74
Apegnin	6.08	1.36

Table (3): Identification of phenolic compounds of dried unripe and ripe sour orange peel:

Samples / Phenolic	Mg/100 gm of sample	
compounds(PPM)	Unripe peels	Ripe peels
Gallic	5.70	6.27
Pyrogallol	3541.46	9456.49
4-amino-benolic	1.26	3.88
Protochatchuic	1.26	3.88
Proticathuic	21.11	48.97
Catechein	143.70	68.72
Chlorogenic	21.23	20.42
Catechol	21.18	48.67
Caffeine	103.22	58.45
P-OH-benzoic	104.27	84.62
Caffeic	3.57	13.29
Vanillic	31.363	18.14
P-coumaric	16.93	19.48
Ferulic	174.39	49.49
Iso-ferulic	435.39	239.41
Alpha-Coumaric	-	5.90
Ellagic	73.14	61.75
Benzoic	213.49	80.89
Coumarin	-	24.81
3,4,5 methoxy-cinamic	111.60	22.59
Salycilic	60.22	120.44
Cinnamic	12.54	10.47

Table (4): Effect of supplementation with dried unripe or ripe sour orange peel on feed intake, body weight gain % orangs weight / body weight and peritoneal fat pad % of 25 days of age albino rats:

Groups \ parameter		Feed intake (g/day /each rat)	Body weight gain %	Organs weight / body weight %		Peritoneal fat pad %
				Liver	Kidney	
Control (-ve) group		13.051^{a} ±4.90	228.670^{d} ±4.886	2.641^{e} ±0.102	0.503^{e} ±0.056	2.978^{e} ±0.149
Control (+ve) group fedon (HFD)		13.113^{a} ±0.448	400.543^{a} ±8.630	4.038^{a} ±0.347	0.888^{a} ±0.062	4.436^{a} ±0.088
High fat diet containing	1.5% dried un ripe peel (DURSP)	12.916^{a} ±0.735	356.713^{b} ±16.145	3.781^{ab} ±0.333	0.771^{b} ±0.054	3.910^{c} ±0.080
	3%dried un ripe peel (DURSOP)	13.166^{a} ±0.408	302.413^{c} ±17.633	3.476^{c} ±0.208	0.650^{c} ±0.062	3.456^{d} ±0.080
	1.5% dried rip peel (DRP)	13.333^{a} ±0.408	368.324^{b} ±25.139	3.528^{bc} ±0.178	0.700^{c} ±0.031	4.053^{b} ±0.097
	3% dried ripe peel (DRSOP)	13.583^{a} ±0.736	318.250^{c} ±17.943	3.015^{d} ±0.132	0.571^{d} ±0.050	3.573^{d} ±0.107

Values are expressed as means ± SD.

Values at the same column with different letters are significant at P<0.05

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الخصائص الوظيفية لقشور موالح البرتقال الحامضي وتأثيره على مقاومة السمنة في الفئران

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الملخص العربي

الهدف من هذه الدراسة هو تقدير المكونات الأساسية الموجودة في قشور البرتقال المصريالحامضي الغير تام النضج والتام النضج وتقدير المركبات الفينولية والفلافونات وكذلك دراسة تأثير التدعيم بمستويين من مسحوق هذه القشور على ذكور فئران الألبينو اسبراجو دولى البالغة من العمر ٢٥ يوم، استخدمت في هذه الدراسة ٣٦ فأر اوزانهم (٤٠ ± ٥جم)، تم تقسيم الفئران الي مجموعتين رئيسيتين، المجموعة الرئيسية الأولي (عددها ٦ فئران) تم تغذيتها علي غذاء أساسي واستخدمت كمجموعة ضابطة سالبة. المجموعة الرئيسية الثانية (عددها ٣٠ فأر) تم تغذيتها علي غذاء عالي الدهن طوال فترة التجربة، تم تقسيمهم الي خمس مجموعات كالتالي: مجموعة منهم (عددها ٦ فئران) تم تغذيتها علي غذاء عالي الدهن وتم استخدامها كمجموعة ضابطة موجبة. المجموعات الباقية (٤ مجموعات فرعية) تم تغذيتها على غذاء عالي الدهن ومحتوية علي مستويين (١,٥ أو ٣%) قشور برتقال غير تام النضج أو قشور برتقال تام النضح. أشارت نتائج تحليل المكونات الأساسية لقشور البرتقال المصري غير تام النضج والناضج أن مستويات البروتين، الدهون، الرماد،الالياف، الرطوبة وفيتامين ج كانت (٨,٢٨٣ %,٥,٩١ % ٤,٩٦٧, %١١,٦٣ %,٦,٢٦ % و ٤٨٦,٨٢جزء في المليون) بالمقارنة (١٠,٥٩٧% ٤,٠٧٢,%٣,٩٧٦,% ٩,٨٨,% ,٥,٧٠%,٢٠٢,٦٦جزء في المليون)، علي التوالي. بينما وجد أن محتوى المركبات الفلافونية المستخلصة من قشور البرتقال الغير ناضح وتام النضج اشارت الي وجود ١٨ مركب.تميزت القشور الغير ناضجة بارتفاع محتواها من Naringin,Hespirdin, Apig-6- rhamnose 8-glucose, Rutin and Quercetrinأما بالنسبة للمركبات الفينولية أمكن فصل ١٩ مركب من القشور الغير ناضجة

وعدد ٢١ مركب من القشور الناضجة وقد كان المركب الرئيسي السائد من الفينولات هو Pyrogallol حيث كانت نسبته في قشور البرتقال الناضج ٩٤٥٦,٤٩ مليجرام /١٠٠ جرام، بينما كانت نسبته في قشور البرتقال الغير ناضجه ٣٥٤,٤٦مليجرام لكل ١٠٠جرام ,وقد أظهرت النتائج أن المركبات الفينولية السائدة في قشور البرتقال الحامضي تام النضج هي **Isoferulic , Benzoic , Ferulic, Catechein, P-OH-benzoic, caffeine and 3.4,5-methoxy –cinamic**. وقد أظهرت النتائج البيولوجية ان الغذاء عالي الدهن المدعم بمستويين (٣% أو ١,٥ %) من قشور البرتقال الحامضي التام النضج والغير تام النضج أدى الى حدوث خفض معنويفي النسبة المئوية للزيادة في الوزن، النسبة المئوية لأوزانأعضاء الفئران، ونسبة الدهون في الغشاء البروتوني. وخلصت النتائج الي ان الفينولات العديدة و الفلافونات الموجودة في قشور البرتقال الحامضي الغير ناضج والناضج يمكن أن تساعد في مكافحة السمنة.