International Journal of Innovation and Scientific Research
ISSN 2351-8014 Vol. 16 No. 2 Jul. 2015, pp. 425-432

http://www.ijisr.issr-journals.org/

Antioxidant activity of *Citrus aurantium L. var. amara* Peel from western of Morocco, identification of volatile compounds of its essential oil by GC-MS and a preliminary study of their antibacterial activity

Fatima Zahra ESSADIK[1], Sara HAIDA[1], Abderahim KRIBII[1], Abdelaziz Ramadane KRIBII[1], Khadija OUNINE[2], and Amar HABSAOUI[1]

[1]Department of Chemistry, Faculty of Sciences, University Ibn Tofaïl, Box 133, 14000, Kenitra, Morocco

[2]Department of Biology, Faculty of Sciences, University Ibn Tofaïl, Box 133, 14000, Kenitra, Morocco

ABSTRACT: The essential oil isolated from western Moroccan *Citrus aurantium L. var. amara* peel, by hydrodistillation, is analyzed by Gas Chromatography-mass spectrometry (GC-MS). Fifteen compounds representing 99.86% of total oil are identified. The predominant components in the essential oil are limonene (90.90%), linalool (2.52%), myrcene (1.51%) and β-pinene (1.41%).

Being both dietary and biologically active compounds, flavonoids have attracted much attention of investigations as potent species capable of affecting various biological processes in living organisms. The total flavonoids contents of aqueous and methanolic extracts of bitter orange's peel were found to be 1.43 and 14.82 mg Equivalent Quercetin/g respectively.

Furthermore, the antioxidant activity of aqueous and methanolic extracts of bitter orange's peel is measured using free radical scavenging method with $DPPH^{\bullet}$. In addition, a preliminary study of the antibacterial activity of extracts of bitter orange's peel is tested against three bacterial stains: *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

KEYWORDS: Bitter orange, $DPPH^{\bullet}$, flavonoids, hydrodistillation, free radical scavenging.

1 INTRODUCTION

Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line to defense against radical damage, so the need for them becomes more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure. Therefore, antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods [1]. Isolation and characterization of natural antioxidant having less or no side effects for use in foods or medicinal materials in order to replace synthetic antioxidants has become essential [2].

Citrus aurantium L. (commonly known as sour or bitter orange) has long been used in food industry as an ingredient of liqueurs and marmalades, and its extracts have also been employed in traditional Chinese medicine to activate vital energy and circulation, eliminate phlegm, and disperse stagnation [3]. In this context, several epidemiological studies have associated the consumption of phenolic compounds, and more specifically flavonoids, with lower risks of different types of cancer [4] and cardiovascular diseases [5].

Moreover, bitter orange has a complex chemical makeup, though it is perhaps most known for the volatile oil in the peel. The familiar oily residue that appears after peeling citrus fruit, including *Citrus aurantium L.*, is the essential oil. It gives bitter orange its strong odor and flavor, and accounts for many of its medicinal effects [6].

Besides, infectious diseases cause a large proportion of the health problems in developing countries. Currently, research continues on new safe and effective antibacterial agents obtained from plants as an alternative from of healthcare, and the

Corresponding Author: Fatima Zahra ESSADIK

development of bacterial resistance to currently available antibiotics had led researchers to investigate the antibacterial activity of such plants [7].

The aim of this study is the characterization of the essential oil from the peel of *Citrus aurantium L.* trees growing in the west of Morocco and to investigate the antioxidant and antibacterial activities of its extracts.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

Citrus aurantium L. var. amara is a plant that belongs to the family Rutaceae, it is rich in vitamin C, flavonoids and essential oil. In traditional Chinese medicine, the peel of the immature fruit is used for indigestion, abdominal pain, constipation and dysenteric diarrhea [6]. Bitter orange used in this work is harvested at March 2014 in Kenitra, western of Morocco.

2.2 ESSENTIAL OIL EXTRACTION BY HYDRODISTILLATION

Fresh peel of *Citrus aurantium L.* is washed and subjected to hydrodistillation using a Clevenger-type apparatus for 3 hours to extract essential oil. The essential oil is separated from the aqueous layer with a simple difference in density and stored in sealed vials at low temperature (4° C) until GC-MS analysis.

2.3 IDENTIFICATION OF THE ESSENTIAL OIL COMPOUNDS BY GC-MS

GC Analysis is carried out using a Perkin Elmer chromatogram equipped with an electron impact (EI) ion source and an Rxi-5ms fused silica capillary column (30m x 0.25mm i.d. x 0.25µm). Oven temperature is performed as follows: 60° C for 5min and goes up to 300° C at 2° C/min, then the temperature is kept 10 min at 300° C. Injector temperature 230° C. Helium is the carrier gas set as a constant flow rate of 1 mL/min. Mass spectra are obtained using a clarus SQ8C spectrometer. The relative percentage of the oil constituents was expressed as percentages by peak area normalization.

Most volatile compounds extracted are identified by comparing the retention times (RT) and comparing the obtained mass spectra of the analytes with those of authentic standards from the NIST libraries.

2.4 PREPARATION OF EXTRACTS

The peel (albedo and flavedo) of Citrus aurantium L. is air-dried at room temperature in the shade for 20 days. After drying, the peel is crushed and stored in a tightly closed glass bottle. 20 grams of powder is macerated in 250 mL of methanol for 24 hours under magnetic stirring. The mixture is filtered; the filtrate obtained is extracted with petroleum ether to remove the non-polar part, then the methanol is evaporated to give the methanol extract, while the residue is macerated in distilled water, under the same condition we obtain the aqueous extract.

2.5 PHYTOCHEMICAL TESTS

Aqueous and methanolic extracts so obtained are subjected to preliminary phytochemical screening of secondary metabolites such as tannins, saponins and flavonoids as follows.

2.5.1 TANNINS

To 1 mL of each extract (aqueous and methanolic) is added in 1 mL of distilled water in a test tube. 2 to 3 drops of diluted ferric chloride solution is added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration [8].

2.5.2 SAPONINS

To 1 mL of aqueous and methanolic extract is added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 minutes [9].

2.5.3 FLAVONOIDS

To 5 mL of each extract (aqueous and methanolic) is added 1 mL of concentrated HCl followed by 0.5 mL of magnesium turnings are added. The solution is observed for the appearance of magenta red or pink color after 3 minutes [10].

2.6 QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS

The colorimetric method used for the estimation of flavonoids is that of ZHISHEN et al. [11] with slight changes in total volume of reagents used. 1 mL of the extract (aqueous and methanolic) is mixed with 3 mL of distilled water and 0.3 mL of 5% sodium nitrite and well mixed. After 5 min of incubation, 0.2 mL of 10% aluminium chloride solution is added. Then, after 30 min of incubation in darkness at ambient temperature, 5 mL of sodium hydroxide is added to the mixture. The absorbance was measured at 510 nm with UV-vis spectrophotometer. Total content of flavonoids are calculated from a quercetin (0.05 – 0.5 mg/mL) standard curve and expressed as milligrams quercetin equivalents gram of sample.

2.7 ANTIOXIDANT ACTIVITY

Antioxidant activity of *Citrus aurantium L.* extracts against stable 2,2-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) is determineted by the slightly modified method of SANCHEZ-MORENO [12]. 0.1 mL of the extract (aqueous and methanolic) is mixed at different concentrations with 2 mL of $DPPH^{\bullet}$ sample (0.03 mg/mL) and then incubated in the dark for 30 min. The absorbance is measured at 517 nm. Ascorbic acid is used as standard. The antiradical activity (three replicates per treatment) is expressed as IC50 (concentration required to cause 50% $DPPH^{\bullet}$ inhibition: mg/mL). The total antioxidant activity (%TAA) is expressed as the percentage inhibition of $DPPH^{\bullet}$ radical and determined with the following equation:

$$\%TAA = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where %TAA is antioxidant activity and Abs is absorbance.

2.8 PRELIMINARY ANTIBACTERIAL ACTIVITY

Agar well diffusion assay is carried out to determine the preliminary antibacterial activity of Citrus aurantium L. peel extracts (aqueous, methanolic and the essential oil). One Gram positive bacteria *Staphylococcus aureus*, isolated from laboratory, and two Gram negative bacteria *Pseudomonas aeruginosa* MC1, isolated from the ground water of M'nasra Kenitra (Morocco) by direct plating onto cetrimide agar medium [13], and *Escherichia coli*, isolated from the discharges of abattoirs and domestic wastewater of Kenitra (Morocco) [14], are screened for their susceptibility to bitter orange's peel extracts. In brief, bacterial strains are inoculated into Mueller Hinton nutrient agar and incubated at 37 °C for 18 hours to obtain a fast exponential growth phase culture. Sterile Petri plates are spreader out using a sterile rake. The inoculation is carried out so as to obtain an antibacterial count of about 1 × 108 CFU/mL (Colony-Forming Units/milliliter). The impregnated disks extracts were carefully layered on the surface of the inoculated agar. Similarly, antibiograms made with disks containing antibiotics (Norfloxacin 5 µg, Erythromycin 15 UI, Fusidic acid 10 µg, Tetracycline 30 UI, Sodium cefotaxime 30 µg, Amoxicilline 25 µg and Gentamicine 10 µg) are used for comparison with the results of the tested extract. Petri plates are then incubated at 37 °C for 18 to 24 hours in upright position. Antibacterial activity was evaluated by measuring the zones of inhibition against the tested bacteria.

2.9 STATISTICAL ANALYSIS

The experimental results are expressed as mean ± standard deviation (SD) of three replicates.

3 RESULTS AND DISCUSSION

3.1 YIELD OF ESSENTIAL OIL

A clear yellow volatile oil with a fresh sweet odor is obtained with a yield equal to 0.3%. This works with the results cited in a recent study of sour orange in Tunisia which was obtained with a yield between 0.12 and 0.46% depending on maturity [15].

3.2 CHEMICAL ANALYSIS OF THE ESSENTIAL OIL

GC-MS chromatogram of essential oil is shown in Fig. 1 and its chemical composition is shown in Table 1. The total number of compounds identified in the volatile oil from fresh peel is fifteen representing 99.86% of the total oil. It is mainly consisted of monoterpene hydrocarbons with limonene (90.90%), myrcene (1.51%) and β-pinene (1.41%) as the major components. The oxygenated monoterpens hydrocarbons with contribution of 2.52, 0.88, 0.24, 0.17 and 0.10% constituted the second major portion of the essential oil from fresh peel with limonene, α-terpineol, nerolidol D, terpin 4-ol and cis-linalool oxide respectively. Sesquiterpene hydrocarbons contributed 0.25 and 0.11% with germacarene D as the major component. Terpene esters and sesquiterpene ketones are present at levels of 0.51 and 0.41% respectively with linalyl acetate, geranyl acetate and nootkatone.

Previous compositional studies regarding the peel oil constituents of bitter orange showed similar results proving that limonene was the major volatile compound. Twenty-seven compounds were identified in the Tunisian Citrus aurantium peel essential oil which was dominated by monoterpenes hydrocarbons (93.49%) and limonene was the major constituent (90.25%) followed by α-terpinene (1.10%) [16]. Another study indicated that the volatile oil of bitter orange's peel is made essentially from monoterpenes hydrocarbons which constitute the main class during ripening varying from 71.21 to 94.61% and reaching a maximum at full maturity, then oxygenated monoterpenes were the second class. The volatile composition showed the predominance of limonene which level varied from 67.90 to 90.95% during ripening, with the highest value reached at the maturity stage [15]. Indeed, the essential oil of sour orange from Corsica is almost consisted of hydrocarbons with limonene as major component [17].

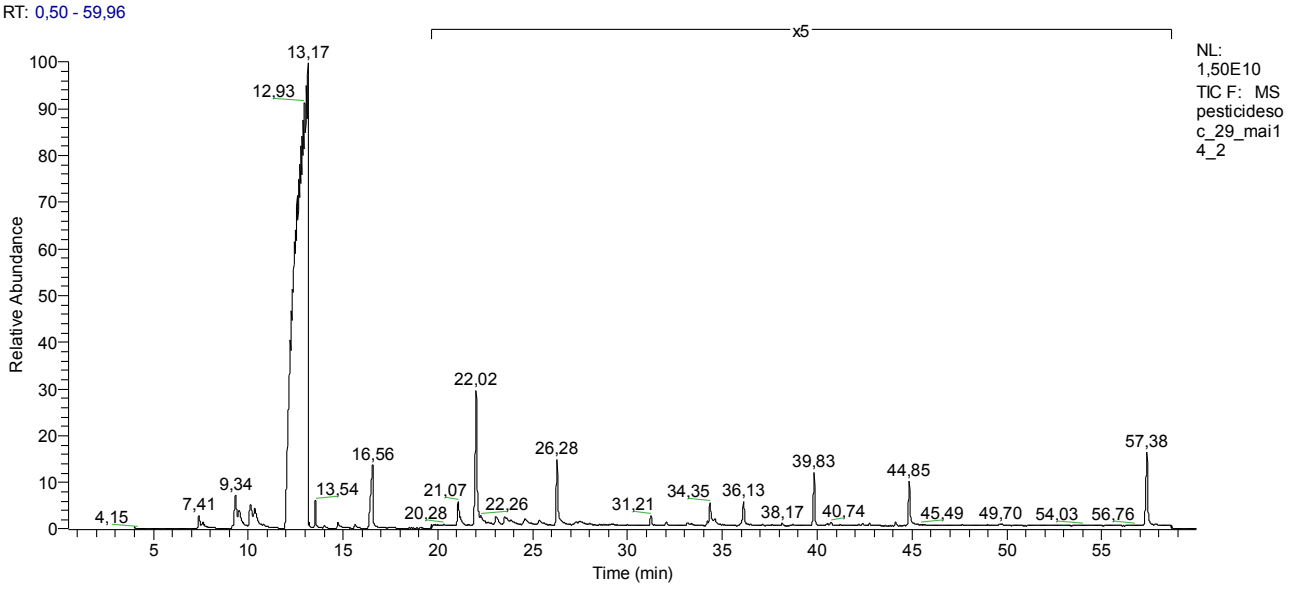

Fig. 1. Chromatogram profile of Citrus aurantium L. peel

Table 1. Essential oil composition of Citrus aurantium peel analyzed by GC-MS.

Volatile compounds	Retention Time (min)	%
α-pinene	7.41	0.43
β-pinene	9.34	1.41
Myrcene	10.11	1.51
Limonene	13.17	90.90
β-ocimene	13.54	0.42
cis-linalool oxide	14.72	0.10
Linalool	16.56	2.52
Terpinen 4-ol	21.07	0.17
α-terpineol	22.02	0.88
Linalyl acetate	26.28	0.39
Geranyl acetate	34.35	0.12
β-Caryophyllene	36.13	0.11
Germacarene D	39.83	0.25
Nerolidol D	44.85	0.24
Nootkatone	57.38	0.41

3.3 PHYTOCHEMICAL SCREENING

Phytochemical investigation of aqueous and methanolic extracts of bitter orange's peel revealed the presence of flavonoids (red color) and the absence of tannins and saponins.

3.4 FLAVONOIDS CONTENTS

Flavonoids are a prominent group of secondary metabolites in citrus fruits that may possess biological activity and have beneficial effects on human health as antimicrobial, anti-inflammatory, anti-diabetic, anti-cholesterolemic, antioxidant and anti-cancer agents [18, 19, 20]. They are reported to possess strong free radical scavenging activities based on their ability to act as hydrogen or electron donors and chelate transition metals [21]. Total flavonoids contents of extracts are expressed as milligram quercetin (a flavonoid present in the natural environement) equivalents gram of sample using the following equation based on the calibration curve: Y= 1.584 x – 0.007, R2= 0.999, where Y is the absorbance and x is the quercetin equivalent (Fig. 2).

The correlation coefficient is to 1 which shows a good linearity of points.

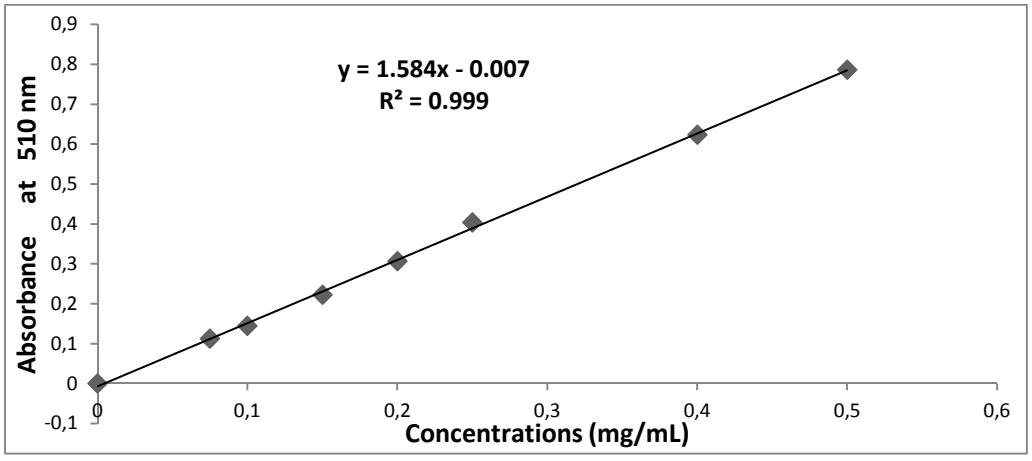

Fig.2. Calibration curve of quercetin

The concentration of flavonoids in Citrus aurantium L. peel is showed in Table 2. Methanolic extract is rich of flavonoids than aqueous extract, this is normal because, according to the procedure of extraction protocol used, methanol allowed us to extract the majority of polar compounds soluble in this solvent, the remaining compounds are extracted by distilled water.

Table 2. Concentrations of flavonoids in aqueous and methanolic extracts expressed in terms of quercetin equivalent (mg of EQ/ g of sample).

Extract	mg of EQ/ g of extract
Aqueous	1.43±0,20[1]
Methanolic	14.82±0.41

[1] *Each value is the average of three analyses ± standard of deviation.*

3.5 ANTIOXIDANT ACTIVITY

The antioxidant activity of aqueous and methanolic extracts of *Citrus aurantium L.* is determined using a methanol solution of $DPPH^{\bullet}$ reagent. $DPPH^{\bullet}$ is a very stable free radical, unlike in vitro generated free radicals such as the hydroxyl radical and superoxide anion, $DPPH^{\bullet}$ has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition. A freshly prepared $DPPH^{\bullet}$ solution exhibits a deep purple color which generally fades when antioxidant molecules quench $DPPH^{\bullet}$ free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the $DPPH^{\bullet}$ molecule) and convert them into a colorless-bleached product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at 517 nm band [22]. The results are summarized in Table 3.

Table 3. Results of $DPPH^{\bullet}$ scavenging activity.

Samples	Equation	R^2 values	IC_{50} values (mg/mL)
Ascorbic acid	y = 501,130x + 1,627	0,992	0,097
Aqueous extract	y = 11,183x + 0,353	0,997	4,439
Methanolic extract	y = 23,634x - 1,827	0,996	2,193

Although it is small, we revealed antioxidant activity compared to ascorbic acid, which is a powerful antioxidant (4.4% for the methanol extract and 2.2% for the aqueous extract of methanol residue). This activity is two times greater for the methanol extract with respect to the aqueous portion from the methanolic residue; this confirms our hypothesis that the extraction of methanol had isolated most flavonoids.

3.6 PRELIMINARY ANTIBACTERIAL ACTIVITY

The antibacterial activity of *Citrus aurantium L.* peel extracts is carried out by the diffusion method on Mueller Hinton agar. Results are shown in Tables 4 and 5. Among the seven antibiotics tested, *Escherichia coli* is sensitive against Gentamicine, *Staphylococcus aureus* is sensitive against Fusidic acid and Norfloxacine, whereas *Pseudomonas aeruginosa* is resistant against all tested antibiotics.

Regarding the extracts, 5 µL of essential oil showed a greater effect than Gentamicine, Amoxicilline and Sodium cefotaxime against *Staphylococcus aureus*, it showed a stronger effect than Sodium cefotaxime against *Peusomonas aeruginosa*. This essential oil has a better effect than all tested antibiotics except Gentaminice against *Escherichia coli*. The methanolic extract has no effect against *Peusomonas aeruginosa*, but has a larger effect than Gentamicine, Amoxicilline and Sodium cefotaxime against *Staphylococcus aureus*, and had a significant effect against *Escherichia coli* than the other antibiotics (except Gentamicine). The aqueous extract has showed no effect against the three strains.

The two Gram negative strains are resistant against the essential oil of *Citrus aurantium L.* from Serbia with 9 mm and 0 mm of diameters respectively, while *Staphylococcus aureus* is sensitive at 14 mm [23]. In a recent study, the methanolic extract of bitter orange from India showed different results from ours, diameters of inhibition against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are 18 mm, 21 mm and 19 mm respectively which are significant [24].

Table 4. Antibacterial activity of tested antibiotics

Tested antibiotic	Load disk	Zone of inhibition (mm)			Critical diameters (mm)		Critical concentrations (µg/mL)
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	Sensible	Resistant	
Gentamicine	10 µg	23	16	8	≥ 15	< 15	6
Amoxicilline	25 µg	NZ	NZ	8	≥ 18	< 18	8
Sodium cefotaxime	30 µg	NZ	8	8	≥ 15	< 15	10
Fusidic acid	10 µg	NZ	NZ	32	≥ 11	< 11	20
Norfloxacine	5 µg	NZ	NZ	26,5	≥ 19	< 19	5
Erythromycine	15 UI	NZ	NZ	NZ	≥ 15	< 15	4
Tetracycline	30 UI	NZ	NZ	NZ	≥ 23	< 23	2

NZ: No zone.

Table 5. Antibacterial activity of Citrus aurantium L. peel extracts against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus

	Load disks	Zone of inhibition (mm)		
		Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
Essential oil	5 µL	9,5	9	9
Methanolic extract	5 µL	10	NZ	11

NZ: No zone.

4 CONCLUSION

In conclusion, our study indicated that Moroccan *Citrus aurantium L.* essential oil's peel is a potential natural source of monoterpenes such as limonene, linalool, myrcene and β-pinene. In addition, as a result of the scavenging and antibacterial effects, it is expected that citrus extracts and the related flavor components may contribute to the prevention of oxidation as antioxidants and free radical scavengers, and the inhibition of some bacteria which have harmful effects on human health. This research may be of interest from a functional point of view and for the valorization of *Citrus aurantium L.* in Morocco and the wider Mediterranean region.

We wanted to explore the part of citrus peel, but pulp is a reservoir of vitamins and polyphenols with beneficial health effects. In next study, we will look at other parts of the plant to assess their biological proprieties (antioxidant and antibacterial) highlighting the synergy phenomenon.

ACKNOWLEDGMENTS

The authors are grateful to CAPM (Centre Anti Poison et Pharmacovigilance du Maroc) for the CG-MS analysis of the essential oil.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

REFERENCES

[1]: A. Kumaran and R. Joel Karunakaran, "Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus", Food Chemistry, vol. 97, no. 1, pp. 109-114, 2006.

[2]: C.K. Ramesh, K.L. Raghu and K.S. Jamuna, "Govinden Soulange Joyce and Ranghoo-Sanmukhiya Vijayanti Mala and Vijay Avin BR. Comparative evaluation of antioxidant property in methanol extracts of some common vegetables of India", Annals of Biological Research, vol. 2, no. 2, pp. 86-94, 2011.

[3]: M. Ou, "Chinese–English manual of common-used in traditional Chinese medicine", Hong Kong: Joint Publishing Co., pp. 348-349, 1989.
[4]: S. N. Nichenametla, T. G. Taruscio, D. L. Barney and J.H. Exon, "A review of the effects and mechanism of polyphenolics in cancer", Critical Reviews in Food Science and Nutrition, vol. 46(2), pp. 161-183, 2006.
[5]: P.M. Kris-Etherton, K.D. Hecker, A. Bonanome, S.M. Coval, A.E. Binkoski, K.F. Hilpert *et al.*, "Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer", American Journal of Medicine, vol. 113, pp. 71-88, 2002.
[6]: Jyotsna A. Saonere Suryawanshi, "An overview of Citrus aurantium used in treatment of various diseases", African Journal of Plant Science, vol. 5(7), pp. 390-395, 2011.
[7]: H.G. Preuss, B. Echard, M. Enig *et al.*, "Minimum inhibitory concentrations of herbal essential oils and monolaurin for Gram-positive and Gram-negative bacteria", Mol Cell Biochem, vol. 272, pp. 29-34, 2005.
[8]: G.E. Trease and W.C. Evans, "Pharmacognosy", 13th edition Bailliere Tindal, London, pp. 278-279, 1989.
[9]: J.B. Harbone, "Phytochemical Methods: A Guide to Modern Technique of Plant Analysis", 2nd ed. Chapman and Hall: New York, NY, 1973.
[10]: J. Parekh and S. V. Chanda, "In vitro antimicrobial activity and phytochemical analysis of some Indian medicinal plants", Turkish Journal of Biology, vol. 31, no. 1, pp. 53-58, 2007.
[11]: J. Zhishen, T. Mengcheng and W. Jiaming, "The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radicals", Food Chemistry, vol. (64), pp. 555-559, 1999.
[12]: C. Sanchez-Moreno, "Methods used to evaluate the free radical scavenging activity in food and biological systems", J. Food science and Technology international, vol. 3, pp. 121-137, 2002.
[13]: S. Bricha, K. Ounine, S. Oulkheir, N. E. El Haloui and B. Attarassi, "Heat resistance of Pseudomonas aeruginosa in preparations at the base of cucmber, tomato and lettuce as affected by pH and sodium chloride", World Journal of Biological Research, vol. 3 issue 1, 8 pages, 2010.
[14]: S. Oulkheir, K. Ounine, N.E. Elhaloui and B. Attarassi, "Survie d'Escherichia coli des conditions acides", Bull. Soc. Pharm. Bordeaux, vol. 145, pp. 41-52, 2006.
[15]: S. Bourgou, F. Z. Rahali, I. Ourghemmi, and M. Saïdani Tounsi, "Changes of Peel Essential Oil Composition of Four Tunisian Citrus during Fruit Maturation", The Scientific World Journal, vol. 2012, 10 pages, 2012.
[16]: I. Jabri karoui and B. Marzouk, "Characterization of Bioactive Compounds in Tunisian Bitter Orange (Citrus aurantium L.) Peel and Juice and Determination of Their Antioxidant Activities", Bio Med Research International, vol. 2013, 12 pages, 2013.
[17]: M.L. Lota, D. De Rocca Serra, C. Jacquemond, F. Tomi and J. Casanova, "Chemical variability of peel and leaf essential oils of sour orange", Flavour and Fragrance Journal, vol. 16, pp. 89–96, 2001.
[18]: J. Vanamala, L. Reddivari, K.S. Yoo, *et al.*, "Variation in the content of bioactive flavonoid in different brand of orange and greape fruit", J. Food Comp. Anal. vol. 19, pp. 157-166, 2006.
[19]: Y.S. Huang, S.C. Ho, "Polymethoxy flavones are responsible for the anti-inflammatory activity of citrus fruit peel", J. Food Chem., vol. 119, pp. 868-873, 2010.
[20]: A.D. Chanet, C. Milenkovic, A. Manach, *et al.*, "Citrus flavanones: whatis their role in cardiovascular protection?", J. Agric. Food Chem. Vol. 60, pp. 8809–8822, 2010.
[21]: K. Le, F. Chiu, K. Ng, *et al.,* "Identification and quantification of antioxidants in Fructus lycii", J. Food Chem., vol. 105, pp. 353-363, 2007.
[22]: R. AMAROWICZ, B.R. PEGG, P. RAHIMI-MOGHADDAM, B. BAR and J.A. WEIL, "Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies", Food Chem., vol. 84, pp. 551-562, 2003.
[23]: M. Sokovic, J. Glamoclija, P.D. Marin, D. Brkic and L.J.L.D. Van Griensven, "Antibacterial Effects of the Essential Oils of Commonly Consumed Medicinal Herbs Using an In Vitro Model", Molecules, vol. 15, pp. 7533-7546, 2010.
[24]: P.M. Tumane, V.G. Meshram and D.D. Wasnik, "Comparative study of antibacterial activity of peel extracts of Citrus aurantium L. (bitter orange) and Citrus medica L. (lemon) against clinical isolates from wound infection", International Journal of Pharma and Bio Sciences, vol. 5(1), pp. 382-387, 2014.