ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: https://ijrps.com

Phytochemical screened, characterization and antibacterial activity of hesperetin and hesperidin extracted and isolated from dried oranges peels

Bassam A. Hassan*[1], Fadil Mohsen Hamed[1], Firas F. Alyaseen[2]

[1]Depatment of Pharmaceutical Chemistry, College of Pharmacy, Thi-Qar University, Iraq
[2]Department of Pharmacognosy, College of Pharmacy, Thi-Qar University, Iraq

Article History:

Received on: 12.05.2018
Revised on: 21.08.2018
Accepted on: 23.08.2018

Keywords:

Antibacterial Activity,
Hesperidin,
Hesperitin,
Orange Peels,
Phytochemical Screen

ABSTRACT

Oranges, fruits are bioactive citrus fruits belong to the *Rutaceae*, family. At the beginning of this study, Phytochemical screened of dried oranges peels showed the absence of coumarins, saponins, sterols, terpenoids, anthraquinones and protein. While the presence of flavonoids, alkaloids, carbohydrate, glycosides, tannins, polyphenol. Afterwards, Hesperetin (flavanone) and hesperidins (flavanone glycoside) were extracted and isolated from dried oranges peels (citrus fruits) and characterized by FTIR spectra, TLC, melting point, Chemical test. In time antibacterial activity of Hesperetin and hesperidins studied against some pathogenic bacterial strains isolated from patients like *Streptococcus, Acinetobacter, E.coli, Klebsiella, Staphylococcus, Aeromonas*. Eventually, Hesperetin (flavanone) showed a Higher biological activity significant than hesperidins and cefuroxime drugs.

* Corresponding Author

Name: Bassam A. Hassan
Email: bassam_org@yahoo.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v9i4.1685

Production and Hosted by

IJRPS | https://ijrps.com

INTRODUCTION

Throughout the eras, plants have been used by the human world as a source of foodstuff, beauty products (cosmetics), medicine, and clothing (even shelter). In addition, plant products also play a vital, role in the health systems care in developed countries, it apparent worldwide agricultural citrus fruit production, like oranges, grapefruits, bergamots, mandarins, Bitter orange, Sindhi, and lemons has been increasing rapidly and strongly in the last decades, reaching more than100 million, metric tons per year (Sharma *et al.,* 2013; Khan *et al.,* 2010).

It was apparent beforehand that the plants of the citrus fruits are recognized as being a healthful source because It possesses bioactive compounds like phenolic compounds, Flavonoids, tannins, carotenoids, vitamins, fibre, naringenin and Hesperidin (Bocco *et al.,* 1998; Gorinstein *et al.,* 2001; Yang *et al.,* 2011). At that point, Hesperidin is the most active compound of orange fruit a polyphenolic flavanones glycoside. Found in the peel and pulps, consisting of the sugar pats called glycone bound to the non-sugar part called aglycone. Glycone parts its disaccharide rutinose and aglycone part called hesperetin as shown in figure 1 (Arora and Kaur, 2013).

Earlier many researchers focus on the biological activity of orange fruit and Hesperidin. At that point, Hesperidin showed as an antioxidant, anti-inflammatory, and anticarcinogenic properties. Moreover, it has been found to reduce significantly, ROS generation in, cells and to restore mitochondrial, enzyme activity (Khan *et al.,* 2014; Castro-Vazquez and Alañón, 2016). At this present literature, we made a comparable biological activity between the of Hesperetin (flavanones) and Hesperidin (flavanones glycosides) against some pathogenic bacterial strains isolated from patients like *Streptococcus, Acinetobacter, E.coli, Klebsiella, Staphylococcus, Aeromonas* were done.

Finally, Hesperetin flavanones showed a Higher pharmacological activity significant than Hesperidin flavanones glycosides and cefuroxime drugs as summarized in table 4.

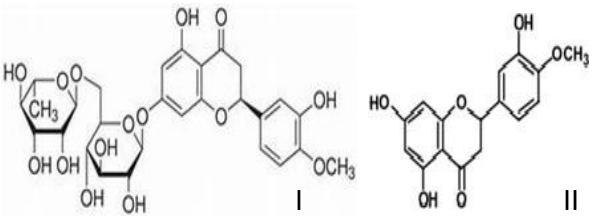

Figure 1: Chemical structure of Hesperidin I and Hesperetin II

MATERIAL AND METHODS

Materials

The orange fruit was collected, from AL Nasiriya local market were peeled then washed using water tap then twice using distilled water. Then the peel of orange was dried and grounded, properly by using mortar, and pestle then using a grinder machine of seasons to fine. Powder for further use. All chemicals obtained from the college laboratory. The work was performed at the chemistry lab.

Qualitative phytochemical Screened

Plants, considerable bioactive chemical, components that produce specific physiological and, biochemical activity in the human body.

These bioactive constituents are glycosides, alkaloids, tannin, flavonoids, coumarins, sterols, phenolic compounds, etc. (Chede 2013; Lawal *et al.,* 2013). To determine the phytoconstituents of ethanol extract prepared by, Soxhlet 10 grams of Orange peel powder in 100 mL of ethanol for 1hrs. The ethanol extract was filtered through a Buchner funnel, then concentrated one, to fifth using a rotatory evaporator under vacuum at a temperature of 450°C. Then collected to, confirmed the phytoconstituents present. As shown in table 1.

Test for alkaloids: 1 ml of Dragendroff's, reagent and two drops of HCl was added to the to the sample solutions. Formation of reddish brown precipitate indicated the presence of alkaloids

Test for flavonoids: Alkaline., reagent test to the sample (solutions, a few drops of sodium, hydroxide solution were, added. Formation of intense yellow colour, which, turned colourless after addition, of few drops of dilute hydrochloric acid, indicated the presence of flavonoids.

Test for carbohydrates: Small amount of Molisch's, reagent mixed with few drops of, concentrated sulphuric acid was added slowly down the sides of the sloping test tube. A purple, ring at, the junction appeared indicated the presence, of carbohydrates.

Fehling's test: a small, quantity of Fehling's solution is mixed with the sample, solutions and heated red, a precipitate appeared to, indicated the presence of reducing sugar.

Test for tannins and, polyphenols: Small amount of the sample solutions, was dissolved in distilled water and 10% of lead, acetate solution was added to a mixture, a white precipitate indicated the presence of phenolic, and tannins.

Resins test: a small amount, of the sample solution, are mixed with 95% ethanol and 4% HCl. Turbidity not appeared.

Coumarines test: Few drops of sample solution added to the filter paper soaked by diluted NaOH. the yellowish green colour on filter paper not appeared

Test for saponin: A small, quantity of the sample was diluted with 15 mL of distilled water and shook vigorously; formation of 1 cm layer of foam which is stable for 10 min indicated the presence of saponins.

Test for sterols: Chloroform, was added to the sample solutions followed by few drops, of Liebermann-Burchard Test or, Acetic Anhydride Test and concentrated sulphuric acid along the slop sides of the tube. A green colouration not appeared indicated the absence of sterols.

Test for terpenoids: Chloroform was added to the, sample solutions followed by a few drops of, concentrated sulphuric acid along the slop sides of the tube, (Salkowski reaction). A red-brown, colouration not appeared indicated the absence of terpenoids.

Test for anthraquinone glycosides: 1 mL of the samples, a few drops of 10% potassium, hydroxide solution were added. No formation of red colour so not confirmed the presence of anthraquinone glycosides.

Test for Proteins: A solution of ninhydrin, in ethanol, is added, to the sample solutions. A purple colour not appeared, indicated the absence of Proteins and amino, acids (Suja *et al.,* 2017; SPANDANA *et al.,* 2016).

Extraction of hesperidin flavonoid glycoside:

Cold extraction, 50 gm. of orange peel powder, macerated in 250 ml of petroleum ether, two days. Then filtrated through a Buchner funnel defatted process to remove non-polar components, like resins aromatic oil, fatty acid and waxes etc. Then cooled and dried.

Table 1: Phytochemical screening

Chemical structure	Chemical test	Test Result
Alkaloids	Wagner reagent	+ ve
Flavonoids	Shinoda test	+ve
Carbohydrates	Molish test	+ve
Glycosides	Fehling's test	+ve
Tannins or Polyphenols	10% of lead acetate	+ve
Resins	Ethanol 95% +boiling + 4% HCl	+ve
Coumarins	Filter paper soaked by diluted NaOH	-ve
Saponin	Shaken of the extraction	-ve
Sterols	Liebermann Burchard	-ve
Terpenoids	Salkowski reaction	-ve
Anthraquinones	Borntrager's test	-ve
Protein	Ninhydrin test	-ve

(where, – absent and + present)

Table 2: Phytochemical screening of Hesperitin and Hesperidin (where – absent and + present)

Sample Name	Molish test	Fehling's test	Shinoda test	Alkaline test
Hesperidin	+ve purple ring	+ve red ppt	+ve Crimson	+ve Colorless
Hesperitin	-ve	-ve	+ve Crimson	+ve Colorless

Hot extraction, after complete cold extraction, (maceration) the contents were heated under, Soxhlet for 3 hours with 300 mL in of 90 % ethanol (40 – 60°C) to isolate the polar constituents like flavonoids, glycoside etc. The filtrate was concentrated on the small volume by rotary evaporator. Then acidifying (pH 3-4) with 20 ml of 6% acetic acid. Keep the concentrated residual liquid in a refrigerator (4-6°C) overnight when a solid crystalline substance appears then washed with cold distilled water then dried to get 2.9 gm of hesperidin. Physical properties like Color, odour, melting point and, yield, are listed in table 1.

Isolation of Hesperitin from Hesperidin

A mixture of hesperidin (2 g) and, methanol (50 mL) and 50 % of Hydrochloric acid (2 mL) was, stirred and heated for 1 hour. The resulting, homogeneous solution was concentrated, one to fifth then cooled and transferred to a separator, funnel, shake with15 ml of chloroform, twice then separated. The chloroform, layer evaporated to dryness to give the product Hesperitines. Hesperetin was purified by the following, procedure: Dissolve the crude product in a minimum of acetone, and the resulting solution was added, to a vigorously stirred mixture, of water (200 mL) and acetic acid (3 mL). In an ice bath, precipitated hesperetin was washed and cooled, with water to get 1.2 gm. Physical properties listed in a table No3 (Lahmer and Belboukhari, 2015).

RESULTS AND DISCUSSION

Phytochemistry of Hesperitin and Hesperidin

After Hesperitin, and Hesperidin isolated, from orange peel, by extraction and isolation, method mentioned above, it was identified, chemically to confirm the isolated Hesperitin and Hesperidin by dissolved few amounts of the crystals, in one ml of ethanol mixed in the test, tube by reagents like Molish test (for general carbohydrate), Fehling's test (for glycosides), Specific test for flavinoids Shinoda test and, Sodium hydroxide test.

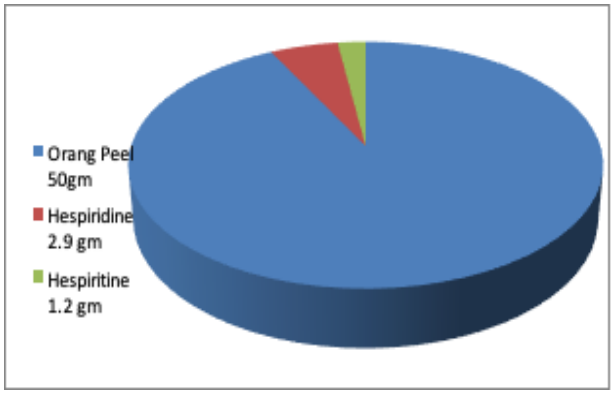

Figure 2: Graphical representation of Hesperidin and Hesperitin yield

Hesperidin showed a positive result with Molish test and Fehling's test for glycoside and also showed, positive Shinoda test and, alkaline test for flavanones respectively. While Hesperetin showed a negative result with Molish test and Fehling's, test for glycoside and also, showed positive Shinoda test and alkaline test respectively. That is results indicate Hesperetin (flavanones) and Hesperidin (flavononesglycoside) were isolated from oranges peels.

Chromatographic analysis results (TLC): Hesperidin extracted from orange, peel analyzed over Silica gel, eluted with, butanol: acetic acid: water

(4:1:5) Rf = 0.61. Hesperetin was chromatographed over Silica gel, eluted with methanol: chloroform (9.0: 1.0) Rf = 0.72. Which were observed, close to the standard Rf value of commercial Hesperidin (0.53) and hesperetin (0.66) from the literature (Lahmer and Belboukhari, 2015).

Melting point

Melting point /SMP3I, apparatus. A melting, point of Hesperidin 254°C, While, Melting point of Hesperitin 226°C, which was appeared close to the melting point of Hesperidin and Hesperitin 257°C, 221°C from the literature.

Table 3: Physical Characters of Hesperidin and Hesperitin

Parameter	Observation Hesperidin	Observation Hesperitin
Colour	Yellowish brown	Yellow
Odour	Aromatic	Aromatic
Melting Point	254°C	226°C
Yield	5.8%	2.4 %
Rf	0.61	0.72

FTIR Spectral analysis

The FTIR spectra in the range (4000-200) cm-1 were, recorded as CsI discs using a Shimadzu, FTIR spectrophotometer. The FTIR, spectrum for Hesperetin shows a characteristic, stretching absorption band. 35091cm-1 (O-H str.), 3055cm-1 (C-H str. (arene)), 2947cm-1 (CH str., (alkane), 2839 (CH str. (alkane–OCH3), 1642 (C=O Str.), 1612 (Aromatic C=C str.) as shown, in figure 3.

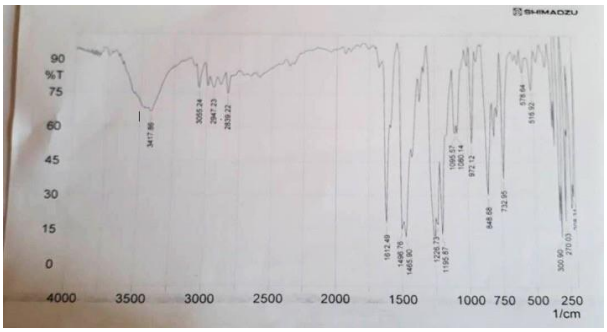

Figure 3: FTIR Spectra of Hesperetin

The previous results of phytochemistry-, TLC and melting point and FTIR spectra showed good results to indicate the Hesperetin (flavonones) and Hesperidin (flavonones glycoside) were extracted and isolated from oranges peels.

Minimum Inhibition concentration solution preparation

The MIC solution of Hesperitin and Hesperidin it was, done by dissolving 0.4 gm in 10 ml of ethanol to get a 40 mg/ml which was the Minimum Inhibition, concentration tested as shown in the table (No3). Sterilization was done by, filtration wares through a Millipore 0.45 mm and 0.22 mm.

Biological activity

In vitro antibacterial activity of Hesperitin and its Hesperidin against some pathogenic bacterial, strains isolated from, patients using agar cup method. (*Streptococcus, Staphylococcus, Acinetobacter, Aeromonas, E.coli and Klebsiella*) (Asia and Aamir, 2015). The results are summarized in table 3.

Table 4: The effect of Hesperidin, Hesperitin and Cefuroxime on the growth of bacteria in vitro against some pathogenic bacterial strains isolated from patients

Types of the bacteria	Diameter zone of inhibition (mm) of Antimicrobial Agent		
	Hesperetin	Hesperidin	CEFUROXIME
Streptococcus	33	19	9
Acinetobacter	32	0	12
E.coli	30	14	2
Klebsiella	22	0	7
Staphylococcus	21	0	18
Aeromonas	19	18	11

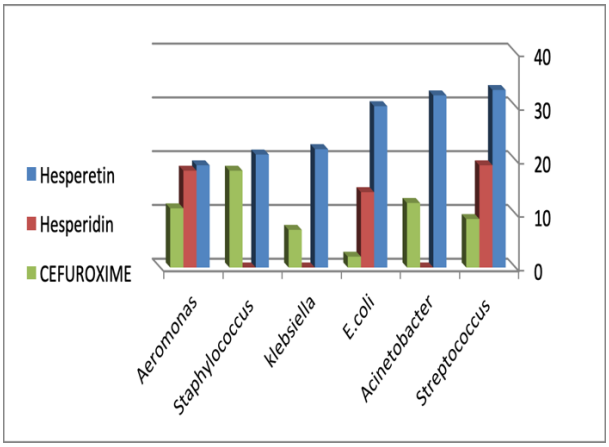

Figure 4: Graphical representation between of Hesperetin, Hesperidin and CEFUROXIME on the growth of bacteria in vitro against some pathogenic bacterial strains isolated from patients

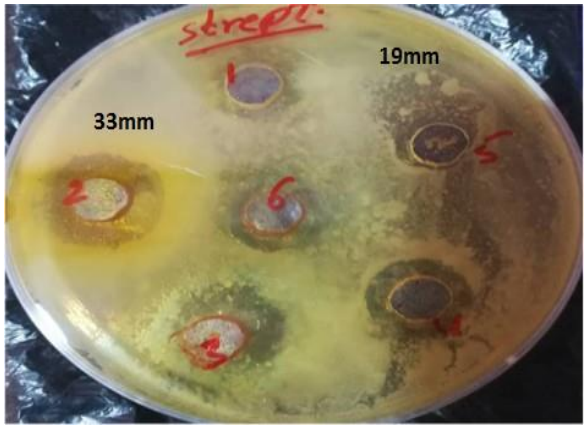

Figure 5: Mean zone, inhibition (mm) of all extracts of Hesperetin and Hesperidin on *Streptococcus* on Muller Hinton Agar

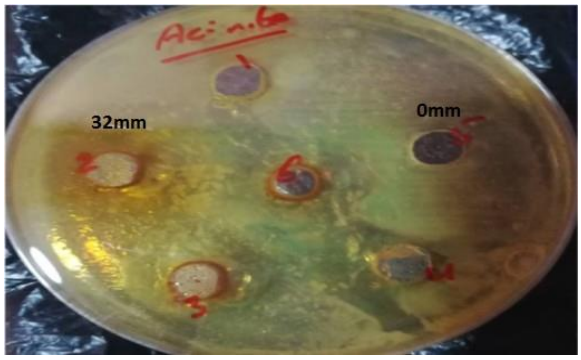

Figure 6: Mean zone of inhibition (mm) of all extracts of Hesperetin and Hesperidin on *Acinetobacter* on Muller Hinton Agar

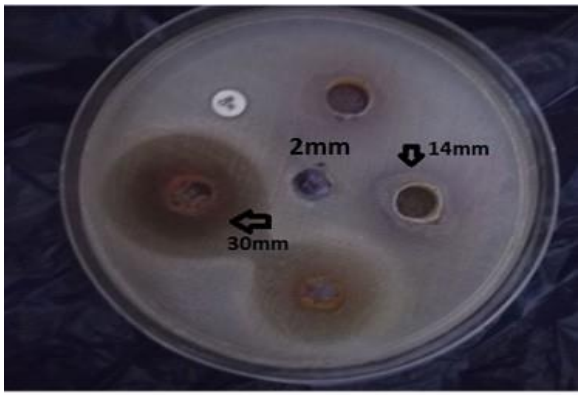

Figure 7: Mean zone of inhibition (mm) of all extracts of Cefuroxime Hesperetin and Hesperidin *E. coli* on MacConeky agar

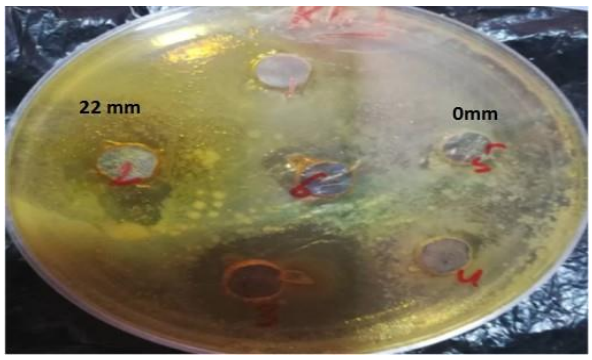

Figure 8: Mean zone of inhibition (mm) of all extracts of, Hesperetin and Hesperidin on *Klebsiella* on Muller Hinton Agar

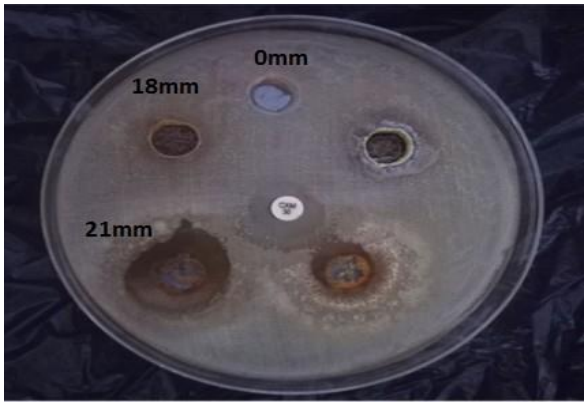

Figure 9: Mean zone of inhibition (mm) of all extracts of Cefuroxime, Hesperetin and Hesperidin *Staphylococcus* on MacConeky agar

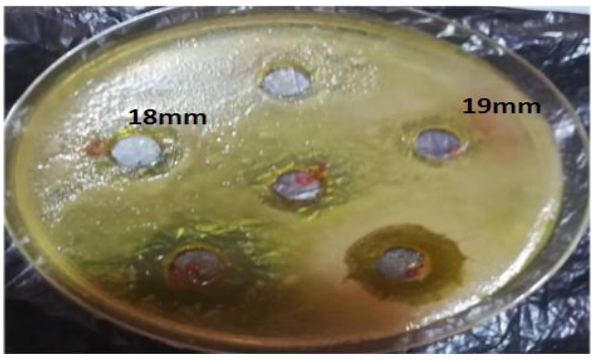

Figure 10: Mean zone of inhibition (mm) of all extracts of Hesperetin and Hesperidin on *Aeromonas* on Muller Hinton Agar

CONCLUSIONS

As discussed previously, Phytochemical screened, of dried oranges peels shown, the absence of coumarins, saponins, sterols, terpenoids, anthraquinones and protein., while the presence of flavonoids, alkaloids, carbohydrate, glycosides, tannins, polyphenol. Afterwards, Hesperetin (flavanone) and hesperidins (flavanone glycoside) were extracted and isolated from dried oranges peels (citrus fruits) and characterized by FTIR spectra, TLC, melting point, Chemical tests like Shinoda and alkaline test as mentioned in the results. Antibacterial, the activity of Hesperetin, Hesperidins, and cefuroxime tested against some pathogenic bacterial strains isolated from patients like *Streptococcus, Acinetobacter, E.coli, Klebsiella, Staphylococcus, Aeromonas*.

In the end, Hesperetin flavanones were isolated from the oranges peels and identified the biological activity test showed Hesperetin (flavanones) a Higher biological activity significant than, Hesperidin (flavanones glycosides) and cefuroxime drugs as summarized in table No 4.

REFERENCES

A. Bocco, M.-E. Cuvelier, H. Richard, and C. Berset.1998. "Antioxidant activity and phenolic composition of citrus peel and seed extracts" Journal of Agricultural and Food Chemistry, vol. 46, no. 6, pp. 2123–2129.

Adewole E, *et al.,* 2014. Phytochemical Constituents and Proximate Analysis of Orange Peel (Citrus Fruit). J. Of Advanced Botany and Zoology, V1I3. DOI: 10.15297/JABZ.V1I3.02.

Chaudhry VK, Hussain Z, Pandey A, Khan R, Srivastava AK. 2016. Isolation and characterization of hesperidin from the dried orange peel, International Journal of Research in Pharmacy and Science, 6 (2); 15 –18.

Chede PS. 2013. Phytochemical analysis of Citrus sinensis peel. Int J Pharm Biol Sci.4 (1): 339-343.

Lawal D, Bala JA, Aliyu SY, Huguma MA. 2013. Phytochemical screening and in vitro anti-bacterial studies of the ethanolic extract of Citrus sinensis (Linn.) Peel against some clinical bacterial isolates. Int J Innov Appl Stud 2 (2): 138-145.

Lucia Castro-Vazquez, María Elena Alañón.2016. Bioactive Flavonoids, Antioxidant Behaviour, and Cytoprotective Effects of Dried Grapefruit Peels (Citrus paradisi Macf.), Hindawi Publishing Corporation, Volume 2016 Article ID 8915729, 12 pages.

M. K. Khan, M. Abert-Vian, A.-S. Fabiano-Tixier, O. Dangles, and F. Chemat. 2010. "Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (Citrus sinensis L.) Peel," Food Chemistry, vol. 119, no. 2, pp. 851–858.

Mamta Arora, Parminder Kaur. 2013. Antimicrobial & Antioxidant Activity of Orange Pulp and Peel, International Journal of Science and Research (IJSR), Volume 2 Issue 11, November.

Muhammad Kamran Khan, Zill-E-Huma, Olivier Dangles.A. 2014. A comprehensive review on flavanones, the major citrus polyphenols, Journal of Food Composition and Analysis, Volume 33, Issue 1 February, Pages 85-104.

N. Lahmer, N. Belboukhari. 2015. Hesperidin and hesperetin preparation and purification from Citrus sinensis peel, A. Cheriti and K. Sekkoum, Der Pharma Chemical, 7 (2):1-4.

Omm-e-H, Asia N and Aamir A**.** 2015. Antimicrobial and antioxidant activities of orange peel (Citrus sinensis) extract. Bulletin of Environment, Pharmacology and Life Sciences. Screening of Phytochemical Constituted, Vol 4 [6] May: 102-108.

Prabhakar Sharma, Prakash Pandey, Ramchandra Gupta, Sunil Roshan, Ashish Garg, Ajay Shukla, Anil Pasi.2013. Isolation and characterization of hesperidin from orange peel, Indo American Journal of Pharmaceutical Research, 3 (4).

S. Grinstein, O. Mart´ın-Belloso, Y.-S. Park *et al.,* 2001. "Comparison of some biochemical characteristics of different citrus fruits," Food Chemistry, vol. 74, no. 3, pp. 309–315.

Spandana U, Sreedevi A, Havilah Esther S, Lakshmaiah P. 2016. Effect of hesperetin isolated from orange peels on cisplatin-induced nephrotoxicity, International Journal of Pharma and Bio Sciences, Oct; 7 (4): (P) 15 – 21.

Suja D, *et al.,* 2017. Phytochemical Screening, Antioxidant, Antibacterial Activities of Citrus Limon and Citrus Sinensis Peel Extracts. Int J Pharmacogn Chinese Med. 1 (2): 000108.

X. Yang, S.-M. Kang, B.-T. Jeon *et al.,* 2011. "Isolation and identification of an antioxidant flavonoid compound from citrus processing by-product," Journal of the Science of Food and Agriculture, vol. 91, no. 10, pp. 1925–1927.