Iraqi Journal of Market Research and Consumer Protection
(2025) 17(2): 192-200.
DOI:
Al-tamimi & AL-Janabi

المجلة العراقية لبحوث السوق
وحماية المستهلك

STUDY OF SOME BIOLOGICAL ACTIVITIES OF RESVERATROL FLAVONOID FROM GRAPE JUICE RESIDUE

Hajir F. N. Al-tamimi [1*], Nidhal M.S. AL-Janabi [2]

[1]Department of Food Science, Agricultural Engineering Sciences College, Baghdad University, Baghdad, ,Iraq .Hajar.Firas2102m@coagri.uobaghdad.edu.iq
[2]Professor PhD., Department of Food Science, Agricultural Engineering Sciences College, Baghdad University, Baghdad Iraq. Nidhal.m@coagri.uobaghdad.edu.lq

Received 2/ 10/ 2023, Accepted 29/ 4/ 2024, Published 30/ 9/ 2025

ABSTRACT

The flavonoid resveratrol was extracted from grape juice residue by boiling water and Crystalized the extract and resveratrol crystals obtained from it have an effect in the growth of some bacteria that transmit diseases through Food the resveratrol crystals(RC) superiority on the extract in inhibiting the growth of gram positive bacteria *B. subtilis* and *S. aureus* excepting *B.cereus* with inhibition diameter rate(32,31.5 and 27.5)mm for crystals and 28,26 and 38.5)mm for aqueous extract (AE) respectively, gram negative bacteria were more sensitive to the extract than RC with diameter (28.5 and 14.5)mm for *E.coli* o157:H7 and for *S.typhimurium* (16 and 8.5)mm respectively while effect of crystals was higher on the growth of *E.coli* 28.5mm tested yeast *C.albicans* and *S.cerevisiae* sensvonoids**itivity to crystals higher than extract 33mm for both of them, RC and extract have an antioxidant effect characterized by its reducing power 90.27% and 92.64% and free radicals scavenging 92.60% and 92.29% respectively.**

Key words: fla, resveratrol free radical scavenging reducing power antimicrobial**.**

دراسة بعض الفعاليات الحيوية لفلافونويد الريسفيراترول من بقايا عصير العنب

هاجر فراس ناظم التميمي[1]، نضال محمد صالح الجنابي [2]
[1]قسم علوم الاغذية، كلية علوم الهندسة الزراعية ، جامعة بغداد ، بغداد ، العراق، Hajar.Firas2102m@coagri.uobaghdad.edu.iq
[2] الاستاذ الدكتور ، قسم علوم الاغذية، كلية علوم الهندسة الزراعية، جامعة بغداد، بغداد، العراق، Nidhal.m@coagri.uobaghdad.edu.lq

الخلاصة

تم استخلاص فلافونويد الريسفيراترول من بقايا عصير العنب بواسطة الماء المغلي وبلورته,وكان للمستخلص وبلورات الريسفيراترول المستحصل عليها منه تأثير على نمو بعض البكتريا التي تنقل الأمراض عن طريق الغذاء، إذ تفوقت بلورات الريسفيراترول على المستخلص في تثبيط النمو بالنسبة للبكتريا الموجبة لصبغة غرام *B.subtilis* و بكتريا*S.aureus* باستثناء *B.cereus* بمعدل قطر تثبيط (32,31.5 و 27.5) ملم للبلورات و(28,26 و 38.5) ملم للمستخلص المائي على التوالي, كانت البكتريا السالبة لصبغة غرام أكثر حساسية للمستخلص من بلورات الريسفيراترول بقطر (28.5 و 14.5) ملم بالنسبة لـ *E.coli* O157:H7 و *S.typhimurium* (16 و 8.5) ملم على التوالي بينما كان تأثير البلورات أعلى في نمو بكتريا *E.coli* 28.5 ملم وحساسية الخميرة المختبرة *C.albicans* و*S.cerevisiae* للبلورات أعلى من المستخلص 33 ملم لكل منهما. RC والمستخلص لهما تأثير مضاد للأكسدة يتميز بقوة أختزالية 90.27% و92.64% وكابحة للجذور الحرة 92.60% و92.29% على التوالي.

الكلمات المفتاحية : فلافونويدات، ريسفيراترول، كبح الجذور الحرة، القوة الاختزالية، مضاد للاحياء المجهرية.

---

[*] **The article is taken from the master's thesis of the first researcher.**

INTRODUCTION

Resveratrol is a natural polyphenols phytochemical (3,5,4′-trihydroxystilbene) with a variety of bioactivities associated with health promotion (**King *et al.*, 2006**), it consist of two aromatic rings connected by an ethylene bond (**Hussein, 2011**). Resveratrol is found in more than 70 plant species especially grape seeds and peels (**Lastra & Villegas, 2007;Salehi *et al*, 2018**), Resveratrol was first identified in grape (*Vitis vinifera*) in 1976 (**Langcake & Pryce, 1976**),in skins of grapes not in the flesh **(Creasy &Coffee, 1988),** the concentration of resveratrol in grape skin and grape variety also effects its concentration ,higher concentration observed in red grape compared to white grape (**Sieman & Creasy, 1992**). Resveratrol has two phenol rings linked together by an ethylene bridge as shown in Figure 1.Doubts are being raised about industrial antioxidants due to their toxic and carcinogenic effects this has led to increasing interest in safer antioxidants which are natural antioxidants in plants for nutritional application, resveratrol has been identified as the active compound of phytoalexin, which contains antioxidant properties (**Gülçin, 2010**).Its polyphenolic composition gives it antioxidant and low density lipoprotein (LDL) activity(**King *et al.*, 2006: Lastra & Villegas, 2007**). Resveratrol is a natural product that affects a wide range of cells and has the ability to inhibit the growth of some pathogenic microorganisms, gram positive and negative bacteria and fungi (**Sharifi-Rad, 2022**),it changes the bacterial expression of virulence traits, leads to decrease in their toxicity and inhibits the formation of biofilms and their decreased mobility in combination with traditional antibiotics (**Vestergaard *et al.*, 2019**).Resveratrol improves the therapeutic results for patients with diabetes, obesity and colorectal cancer, this flavonoid compound has a cancer preventive ability as it has recently been proven to properly prevent the carcinogenesis process, metabolic syndrome high blood pressure ,cardiovascular and kidney disease and infection's (**Singh *et al.*, 2019**).Recently it has been found ,it has protective effects against obesity and neurodegenerative diseases such as Alzheimer and its effective in treating osteoporosis in the post-menopausal period in women and reducing the incidence of breast cancer (**Abd-AL-Hussein, 2021**).

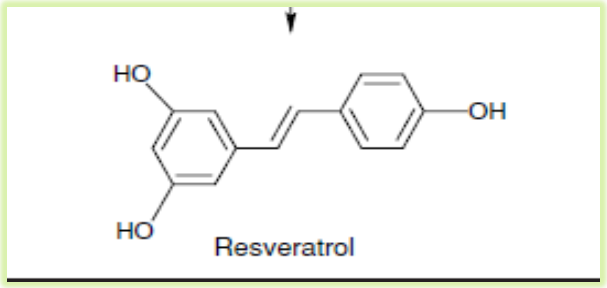

Figure (1): The chemical structure of resveratrol. (**Vestergaard *et al.*, 2019**))

MATERIALS AND METHODS

Preparation of aqueous extract (RWE)

To 10 gm of grape juice waste obtained from one of the shops selling grape juice in Baghdad, add 150 ml of boiling distilled water is added and left for half an hour on the magnetic stirrer Then filtered by using filter paper (whatmanNo.1) and concentrated with a rotary stirrer at a temperature less than 70C^{o} , The concenterated extract is poured into petri dishes to dry in the hot air oven(40-55)C^{o},and scraped the powder the stock solution is prepared at a concentration 100 mg/ml from the powder and sterilized using millipore (0.45µm) and keep it until use.

Preparation of resveratrol crystals (RC)

Fifty ml of the aqueous extract is taken and 50ml of absolute ethyl alcohol is added to it using a separating funnel, the solvent layer is transferred to the rotary evaporator(50C^{O}) to concentrating it, then is placed in the oven at 50C^{o} the crystals of the compound are collected to be tested. The stock solution is prepared at a concentration 100% from the crystals and sterilized using millipore(0.45µm) and keep it until use.

Inhibitory activity against microorganisms

The effectiveness of the aqueous extract of grape juice waste and the crystals extracted from it was estimated against some bacteria and yeasts that cause diseases through food and food poisoning according to what was mentioned in (**Salem & saeed, 2020**).Bacterial cultures are activated in the liquid medium(nutrient broth)at 37C^{O}/18hr and yeasts in the potato dextrose broth(PDA)at 28C^{O}/24hr.

Discs diffusion method

As mentioned in **Abd-ALkarim** (**2022**), both the activated bacteria and yeast were spread 0.1 ml (the concentration cells is compared with the McFerland solution and editing of giving 1.5X10^{8} cell/ml) on the nutrient Agar. made 4 holes with a 6 mm drill bit using a sterile cork drill and placed 100 microliter of extract concentrations and crystals (12.5,25,50 and 100) mg/ml of extract concentrations and crystals. the plates were incubated at 37C^{O}/24hr and 28C^{O}/48hr for bacteria and yeast respectively.

Antioxidant activity

The antioxidant activity of aqueous extract (RWE) and resveratrol crystals (RC) were estimated in two methods:

1- The reducing power:

It was estimated for both the aqueous extract RWE and the resveratrol crystals according to what was mentioned in **Bhalodia *et al.*, (2013)** , by mix 1ml of each separately in different concentrations(10-50)mg/ml with 2.5ml potassium ferricyanide1% and added 2.5ml of phosphate buffer(0.2M,6.6PH) then put mixture in incubator(50C^{O}/20min) after that added 2.5ml TCA10% centrifugation was carried at speed 4000rpm/min, take 2.5ml of the filtrate is mixed with 2.5ml dis. water and 0.5ml ferric chloride 0.1% left the mixture for half an hour, absorbance is measured at a wavelength 700nm.synthetic antioxidant BHT was used and citric acid for comparison. The reducing power was calculated as follows:

$$\textbf{\textit{Reducing power}}\% = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times \mathbf{100}$$

2-Scavenging activity of free radicals

This activity was estimated According to **Al-Jubouri *et al.*,** (**2022**) DPPH is attended at the experience by ethyl alcohol in concentration 0.1 g/ml different concentrations of aqueous extract powder and resveratrol crystals are prepared from (50,100,150,200,250,500,1000 and 1250)µg/ml , by taking 1ml of each concentration and adding 300µl to it. then it was left in the dark for 30min at room temperature after which the absorbance was measured at a wavelength, ascorbic acid was used for comparison while the mixture of the DPPH and ethanol as control the inhibition percentage was calculated according to following equation:

$$\textbf{\textit{Inhibition percentage}}\% = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times \mathbf{100}$$

RESULTS AND DISCUSSION

Antimicrobial activity :It is noted from table(1) that the crystals of the resveratrol compound which we obtained from grape juice waste were characterized by a higher inhibitory effect on gram positive bacteria than the gram negative with an inhibition diameter rate of 32,31.5 and 27.5mm for *Bacillus subtilis* , *Staphylococcus aureus* and *B.cereus* respectively at the highest concentration (100 mg/ml) tested while the diameters of inhibition were lower than for the gram negative bacteria the rate of inhibition reached 28.5 ,14.5 and 8.5mm at the same concentration for *Escherichia coli* this agreed with what has been found by (**Alsaloom, 2023**). In testing biologically active compounds in soybeans, *Escherichia coli* 0157:H7 and *Salmonella typhimurium* respectively, it may be attributed to the difference in the chemical composition of the walls of gram positive and gram negative bacteria. The outer layers of the bacterial wall are different in both types and the resveratrol is weak in penetrating the outer membrane of some gram negative bacteria as they contain exceptional membranes that are not found in gram positive (**Vestergaard *et al.*, 2019**). also it was noted that the four concentrations used were effective on both types of bacteria from the lowest concentration to the highest, the diameters of inhibition zone increase parallel to the increase in the tested concentrations.

Table (1): The inhibitory effectiveness of the aqueous extract and the resveratrol crystals against some types of bacteria and yeasts.

NO.	Name of the microscopic organism	Average diameter of inhibition zone (mm)							
		Concentrations of resveratrol crystals				Concentrations of aqueous (RWE)			
		12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml	12.5 mg/ml	25 mg/ml	50 mg/ml	100 mg/ml
1	*Escherichia coli*	14	22.5	26.5	28.5	-	10	24	26.5
2	*Escherichia coli 0157:H7*	6.5	8.5	12	14.5	-	-	26.5	28.5
3	*Salmonella*	2.5	3	4.5	8.5	-	-	6.5	16
4	*Staphylococcus aureus*	11	25.5	29.5	31.5	-	8.5	22	26
5	*Bacillus subtilis*	16.5	26	32	32	_	21	24	28
6	*Bacillus cereus*	19.5	22.5	26	27.5	_	_	36.5	38.5
7	*Candida albicans*	-	11	30.5	33	-	-	21.5	24
8	*Saccharomyces cerevisiae*	zero	24.5	30.5	33	-	23	28.5	32.5

As for the aqueous extract(RWE) it had a lower effect on the tested bacteria and yeasts than the resveratrol crystals with the exception of the *B.cereus* whose growth inhibition diameter was 38.5mm higher than that of resveratrol crystals ,may be due to the presence of other active compounds in the extract to which the bacteria were more sensitive .The cell wall and cytoplasmic membrane of the cells are damaged by antibacterial substances which leads to the cytoplasm being expelled outside the cell leading to cell death (**Hamaadi, 2017**) *S.aureus* was the least sensitive among the gram positive bacteria with average diameter 26 mm.*E.coli o157:H7* was characterized by being the most sensitive to (RWE) with an inhibition diameter rate 28.5mm higher than their sensitivity to the resveratrol crystals. This didnot agrees with what was found by **(Ibraheem *et al.*, 2018)**. In the inhibitory effect of flavonoids on bacteria. The growth of the two test yeasts was affected by resveratrol with rate of inhibition 33mm for both of them more than it was affected by RWE extract with rate of inhibition 32.5 and 24mm for *Saccharomyces cerevisiae* and *Candida albicans* respectively this is consistent with **(Salim *et al,* 2014)**. effect of total phenols on yeasts. As shown in **Figure 2**.

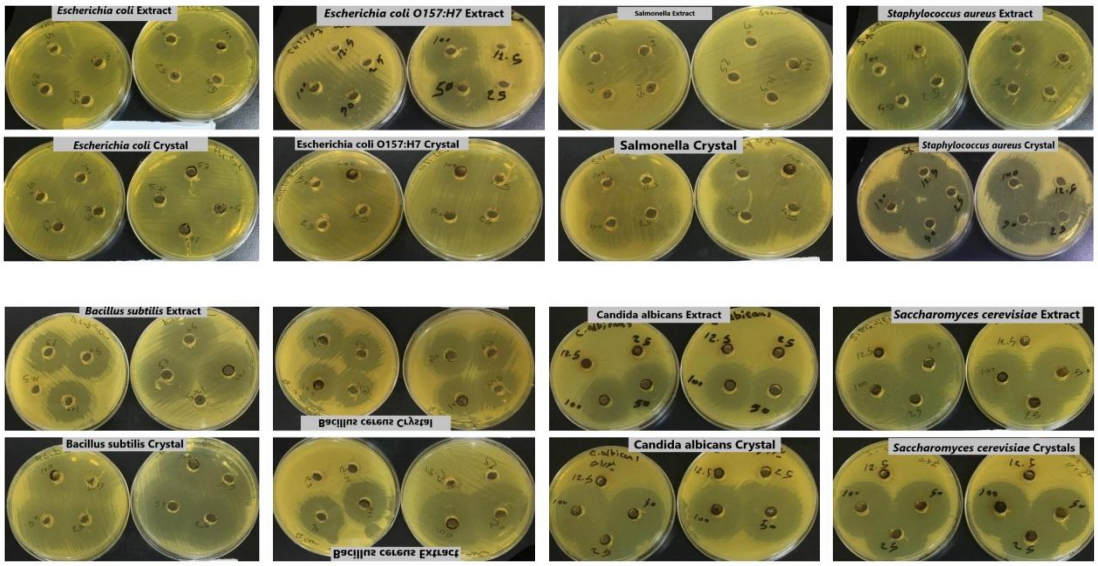

Figure (2): Inhibition zones of the aqueous extract and resveratrol crystals of the tested microorganisms

Reducing power :it is an accurate indicator in antioxidants testing,figure1 shows the superiority of the extract (RWE) and resveratrol crystals over synthetic antioxidant and citric acid with reducing power(92.64 and 90.27)% for extract and resveratrol crystals respectively at the highest concentration used in the experiment(50mg/ml) while the reducing power of the synthetic antioxidant (BHT) was less than 89.22% and less citric acid 87.11% at the same concentration ,noting that the rate of reducing power increases with increasing concentration ,the mechanics of antioxidants vary and flavonoids have a share of this activity as a reductive power this is what **AL-Badri** (**2023**) obtained regarding the effectiveness of the tricin flavonoid at the concentration 50mg/ml as a reductive power that amounted to 93.94% and what **AL-Janabi *et al.,*** (**2013**) found in the extract of grape juice waste from the antioxidant action as a reducing power that was due to its content of total phenolic compounds ,Research results are also consistent with **Hassein** (**2011**) .

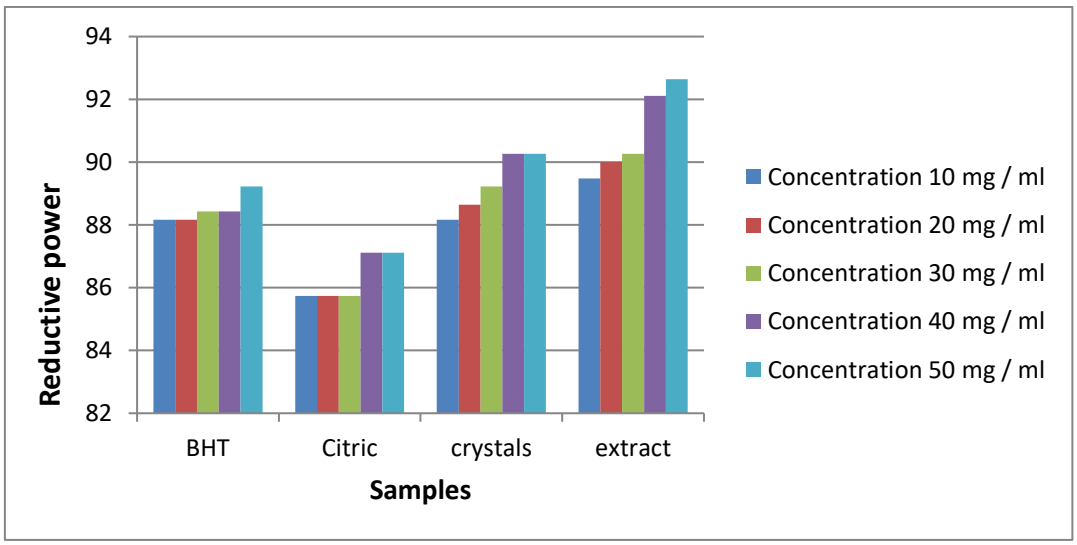

Figure (3): Reducing power of aqueous extract(RWE) of grape juice waste and resveratrol crystals extracted from it.

Free radical scavenging activity :It clear from figure(2) the effect of the extract (RWE) and crystals as an inhibitor of free radicals with the percentage 92.60% for crystals higher than what was recorded in the extract(RWE) 92.29% at the highest concentration (1250µg/ml) tested ,citric acid had a higher share of them as their effectiveness 93.33%.**AL-Wendawi *et al.*,** (**2021**) and **Hassein (2011)** also obtained high effectiveness of citric acid in the free radical suppression test.

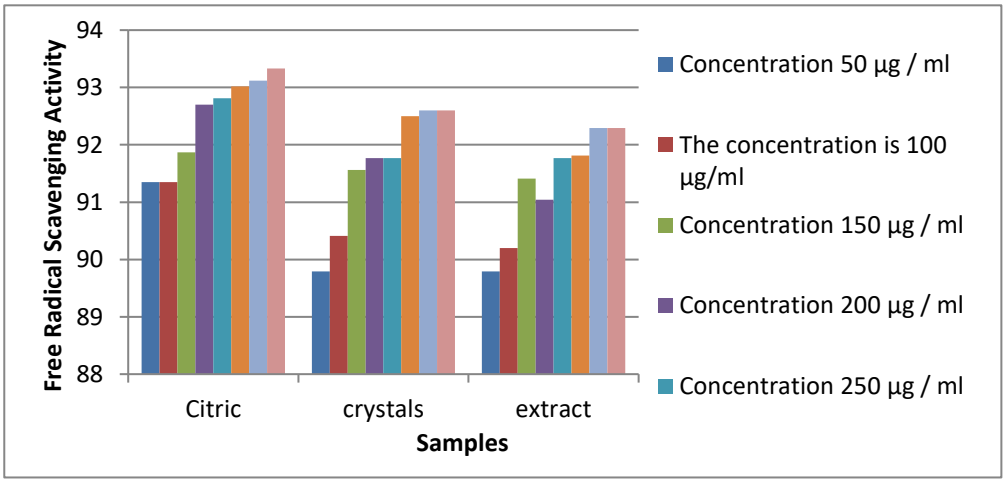

Figure (4): Free radical scavenging activity of extract (RWE) and resveratrol crystals.

CONCLUSIONS

The food industry produces several tons of waste every day ,which puts a burden on the environment ,it also still contains active compounds with various biological activities that can be exploited in food ,pharmaceutical and cosmetic applications .Grape juice waste contains flavonoids in good quantities including resveratrol which has the effect of inhibiting growth ,some microorganisms that transmit diseases through food and delay fat oxidation.

REFERENCES

1. Abd_Alkarim, K. A. (2022). Production of bacteriocins from *lactic acid bacteria* and their role in improving the shelf life of soft cheese. Msc College of Agricultural Engineering Sciences, University of Baghdad.
2. Abd-AL-Hussein, E. S. (2021). The use of resveratrol purified from Grape and industrial residues as an antioxidant in the manufacture of curd. College of Education for Women, Department of Home Economics, University of Baghdad.
3. AL_Badri, Samah, R. H. (2023). Bioactivity of purified tersine from rice bran and its application in some food systems. College of Agricultural Engineering Sciences, University of Baghdad.
4. AL-Janabi, N.M., AL-Samraeem, A.M.M. & Ulaiwi,W.S.(2013).The Effects of Squeezed Grapes Residue on the preservation of Stored fried potato chips. *Pakistan Journal of Chemistry*, 3(1):1-4.
5. Al-Jubouri, A. K., Al-Saadi, N. H. & M. A. Kadhim. (2022). green synthesis of copper nanoparticles from myrtus communis leaves extract: characterizattion, antioxidant and catalytic activity. *Iraqi Journal of Agricultural Sciences*, 53(2): 471-486.
6. Alsaloom, A. N. (2023). Testing and evaluation of bioactive compounds in soybean. *Iraqi Journal of Agricultural Sciences*, 54(1): 85-92.
7. Al-Wendawi, Sh A., and L. A. Gharb. (2021). Antioxidant, antibacterial and antibiofilm potentials of anise (*Pimpinella anisum*) seeds extracted essential oils. *Iraqi Journal of Agricultural Sciences*, 52(2): 348-358.
8. Bhalodia, N. R., Nariya, P. B., Acharya, R. N., & Shukla, V. J. (2013). In vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. *Ayu*, *34*(2), 209–214. https://doi.org/10.4103/0974-8520.119684
9. Creasy, L.L., & Coffee, M.S. (1988). Phytoalexin Production Potential of Grape Berries. *Journal of the American Society for Horticultural Science*,113: 230-4.
10. Gülçin, İlhami. (2010). Antioxidant properties of resveratrol: A structure–activity insight. *Innovative food science and emerging technologies* 11(1): 210-218.
11. Hamaadi, S., R. (2017). Description of compounds suitable for the leaves of the caper plant An effective study in obstetrics. Msc College of Agricultural Engineering Sciences, University of Baghdad .
12. Hussein, M. A. (2011). A convenient mechanism for the free radical scavenging activity of resveratrol. *International Journal of Phytomedicine*, 3.4: 459.

13. Ibraheem, R. M., Mhawesh, A. A. & K. W. Abood. (2018). Estimation of the whole flavonoid, antioxidant, antibacterial challenge concerning viola odorata (banafsha) methanolic extract. *Iraqi Journal of Agricultural Sciences*, 49(4):566-551.
14. King, R. E., Joshua A. B. & David B. M. (2006). Bioactivity of resveratrol. *Comprehensive reviews in food science and food safety*, 5(3): 65-70
15. Langcake, P. & Pryce, R. J. (1976). The production of resveratrol by Vitis vinifera and other members of the Vitaceae as a response to infection or injury. *Physiological Plant Pathology*, 9(1): 77-86.
16. Lastra, C. A., & Villegas, I. (2007). Resveratrol as an antioxidant and pro-oxidant agent: mechanisms and clinical implications. *Biochemical Society Transactions*, 35(5):1156-1160.
17. Salehi, B., Mishra, A. P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., Fokou, P. V. T., Martins, N., & Sharifi-Rad, J. (2018). Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicines*, 6(3): 91. https://doi.org/10.3390/biomedicines6030091
18. Salem, M., & Saeed, M. T. (2020). Potential application of waste fruit peels (orange, yellow lemon and banana) as wide range natural antimicrobial agent. *Journal of King Saud University-Science*, 32(1): 805-810.
19. Salim, Farah D., *et al.* (2014). Determination of total phenol, antioxidant and antimicrobial activities of Avena sativa and Ocimum basilicum. *Baghdad Science Journal*, 11.(2): 1062-1066.
20. Sharifi-Rad, J., Quispe, C., Durazzo, A., Lucarini, M., Souto, E. B., Santini, A., ... & Cruz-Martins, N. (2022). Resveratrol'biotechnological applications: Enlightening its antimicrobial and antioxidant properties. *Journal of Herbal Medicine*, *32*, 100550.
21. Siemann, E. H., & Creasy, L. L. (1992). Concentration of the phytoalexin resveratrol in wine. *American Journal of Enology and Viticulture*, *43*(1): 49-52.
22. Singh, A. P., Singh, R., Verma, S. S., Rai, V., Kaschula, C. H., Maiti, P., & Gupta, S. C. (2019). Health benefits of resveratrol: Evidence from clinical studies. *Medicinal Research Reviews*, *39*(5), 1851-1891.
23 Vestergaard, M., & Ingmer, H. (2019). Antibacterial and antifungal properties of resveratrol. *International Journal of Antimicrobial Agents*, 53(6), 716-723