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Chemical and Biological Evaluation of the Antioxidant In Grape Seed and Skin

By

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7. SUMMARY AND CONCLUSION

The present study was carried out to test some natural antioxidant phenolic compounds from grape by-product (white, red, black skin and white grape seeds) Compared with synthetic antioxidants such as Butylated hydroxytoluene (BHT).

The extracted phenolic compounds were tested as natural antioxidants in more than one experiment conclude chemical and biological evaluation.

Therefore, this study aimed to:

7.1.Chemical evaluation:

1. Preparation three grape varieties skin (white, red and black) and white grape seeds, as provided in season 2010, we used (white Romi, Flame seedless and black Romi).

2. Determination of chemical composition of grape by –product (skin and seeds) each individual and the fatty acid composition of the white grape seeds

3. Determination the phenolic compounds and anthocyanin in grape by-product(skin and seeds).

4. . The phenolic composition of the grape skin and seeds samples was determined by HPLC.

5. Determination the antioxidant activity of grape skin and seeds by DPPH and

Linoleic acid system comparing to synthetic antioxidant (BHT)

6. Measurement of the oxidative stability of sunflower oil at 100 °C by different grape by-products extracts samples in comparing with synthetic antioxidant (BHT).

7.2. Biological evaluation

١. Feeding of albino rats' on basal diet maintain as the control group, Adding the different percentage of grape by-product powder (2, 4. and 8%) to diet and comparing with synthetic antioxidant (BHT) 200ppm.

٢. Recording of organs weight of rats e.g. (liver, kidney, heart and spleen) at the end of experiment.

٣. Determinations of lipid profile, glucose, Liver Enzymes and antioxidant Enzymes.

٤. Investigation of histological changes of livers

The results of our investigation can be summarized in the following points:

****Chemical evaluation results:**

- Chemical composition of white, red, black grape skin and white grape seeds was as follow: moisture (١٨.٩٧, ٧.٢٨, ١٨.٢١ and ٨.٣٨%), fat (٣.٩٣, ٦.٤٢,١.٨٩ and١٠.٣٨%), protein (١٠.١٤٦, ٦.٩٩٩, ٧.٨٧٢ and ٩.٠٧٦%), fiber (١٠.٥٦, ٧.٣٣٥, ٩.٥٦٥ and٣٧.٢٥%), ash (٦.٣٥,٢.٩٤ , ٨.٣٦ and ٢.٣٨%), total Sugar (٥٠.٠٤٤, ٦٩.٠١٦,٥٤.١٠٣ and ٣٢.٥٣٤%) respectively.
- Linoleic acid was the most abundant fatty acid in grape seed oil. It was about 64.72 %of total fatty acids.
- Total phenolic compounds of white, red, black grape skin and white grape seeds were as follow: (٢٩٦.٢٧, ٥١١.٢٣, ٢٠٧٠.٠٢ and ٢٥٣٦.٥ mg/100g) respectively.

- Total anthocyanin of white, red, black grape skin and white grape seeds was as follow: (٤.٠٩, ٤٧.٣, ٣٠٠.٣٧ and ١٣.٦٤ mg/100g) respectively.

- High performance liquid chromatography (HPLC) was used to fraction and identification the phenolic compounds. Catechin was the major phenolic compound presented and identified in grape seeds followed by Procyanidin B1, Di-OH Cinnammic and Procyanidin B3 (٥٢١.٨٠, ٣٥٧.٠١,٢٦٩.٧٠ and 231.87) respectively.

- Seeds extracts showed much higher scavenging activity than all other extracts with EC_{50} 0.259µg extract/µg DPPH followed by black grape skin extract (EC_{50} 3.96µg extract/µg DPPH) while the white grape skin extract showed the lowest scavenging activity with EC_{50} 28.91µg extract/µg DPPH.

- The radical scavenging activity of the grape skin and seeds extracts was dependent upon the contents of phenolic compound.

The effect of addition different grape by-products extraction on oxidative stability of sunflower oil at 100 °C by Rancimat showed that the black grape skin extract have highest oxidative stability (12.9 h) flowed by white grape seed extract and red grape skin extract (12.8 h and 12.8 h) respectively.

****Biological evaluation results:**

The results showed that:

1. There were significant differences ($P \leq 0.05$) in the final body weights of rats in the control group and the remaining groups. The weights of rats fed on standard diet plus investigated grape samples were significantly increase ($P \leq 0.05$) ranged from 50 to 54%.

2. The mean values ± standard deviation of spleen of rats maintained on experimental diets was significantly increase (P≤0.05) than those of the control animals fed only on commercial diet**.**

There were no significant differences in the weight of the other organs (heart, liver and kidney) of rats in all groups.

3. Feed rats on diet containing 200 ppm BHT (synthetic antioxidant) caused significant increase ($P \leq 0.05$) of total cholesterol compared to the control group.

The addition of 2% white grape skin of diet rats caused a significant decrease ($P \leq 0.05$) of total cholesterol compared to the control group. On the other hand, there are no significant different between control group and 4% white grape skin, 8% red grape skin, 2% black grape skin and 8% white grape seed. Meanwhile 4% red grape skin and 8% black grape skin (2, 4%), white grape seed showed that the same effects of BHT.

4. The best results in serum HDL-c recorded for the group fed on diet treated daily with white grape seeds in all levels followed by the group fed on diet treated daily with grape skin 8%, the group fed on diet treated daily with grape skin 4%.

5. Serum LDL-c increased with increasing the level of grape skin in the diet. While adverse action happen in the grape seed 2, 4, 8% (91.22±5.75 ^{bc}, 63.81±11.78^{c}, and 46.12±4.19 ^{h}) respectively**.**

6. Feeding rats on diet containing 2, 4, 8% grape skin (white, red, black) and white grape seed did not affect on VLDL-c in the serum, as compared to the rats fed on basal diet (control group)**.**

7. The results in the same Table illustrated that, synthetic antioxidant and white grape skin at 8% lead to increase serum triglyceride while other treatment did not affect on serum triglyceride

8. Serum Glucose increased gradually by increasing the levels of grape skin and seeds, in the diet. On the other hand, the mean values of serum

Glucose increased significantly in groups which treated with grape skin and seed, comparing with non treated groups with grape**.**

9. Treating rats with different levels of grape skin and seeds led to significant decrease ($P \leq 0.05$) in total antioxidant capacity ,while red grape skin 2 and 4% showed significant increasing level in the same parameter 0.60 ± 0.10^{a}, as compared to the control group**.**

10. Feeding rats groups on diet containing 200ppm BHT, white grape skin, red grape skin, black grape skin at 2% and 4% showed that no significant change ($P \leq 0.05$) in catalase enzyme activity, as compared to the control group. While, feeding rats on diet containing 8% (white grape skin, red grape skin, black grape skin) and white grape seeds at different levels caused a significant increase in catalase enzyme activity.

11. Glutathione enzyme activity in black grape skin 2% and white grape seed 2% groups increased significantly $P \leq 0.05$ (0.06 ± 0.007^{a} and 0.06 ± 0.008^{a}) as compared to the groups fed on the same diets with other different levels of grape skin and seeds.

12. The best results in (AST) and (ALT) were recorded for the groups fed on diet containing 4, 8% grape skin and seed, this treatment decreased liver function (ALT) by about 63.63% than that of the control group.

13. Histopathological investigation of Liver rats fed on different doses of grape skin and seeds indicated that the best result was in the dose 200ppm of white grape skin.