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Original Research Article

Comparative Study, Phytochemical Screening and Antioxidant Activities of Three Types of Apple Seed Extracts

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ABSTRACT

The "Red Delicious, Golden Delicious, and Granny Smith" apples are widely cultivated and consumed worldwide. Because of their therapeutic phytochemical content, research findings have confirmed their beneficial effects in disease prevention and management. The present study was aimed at determining the phytochemical profile and evaluating the antioxidant activity of the seed extract of three varieties of apple. Water, methanol, chloroform, and hexane were used as solvents to extract the seeds of the three varieties of apples. The three crude apple seed extracts were subjected to preliminary phytochemical screening and antioxidant activity testing using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The obtained extracts tested positive for alkaloid, flavonoid, saponin, and tannin compounds in phytochemical screening. However, no glycosides or anthraquinones were observed in any of the extracts. The outcome of the antioxidant capacity test for all the varieties of apple seed extracts indicated by colorimetry that the intensity of colour was reciprocal to concentration. The antioxidant activity of Red Delicious apple extract was in the order of methanol> chloroform > hexane > aqueous extract. However, the antioxidant activity of Golden Delicious apple extract was in the order of chloroform > hexane > aqueous > methanol extract. Furthermore, the antioxidant activity of Granny Smith apple extract was in the order of chloroform > hexane > methanol > aqueous extract. The findings of this study suggest that all the seed extracts from the three apple varieties could be used as new antioxidant compound options.

***Keywords*: A**pple Seed, Antioxidant, Phytochemical Screening, Golden Delicious, Granny Smith, Red Delicious.

Introduction

Herbal science is the study of plant medicinal properties and their applications in health care delivery. There are about 2,500 native plant species in North America, out of a total of 20,000 species that have been used traditionally by Native Americans.[1] Around 80% of the world's population relies on traditional medicine derived from medicinal plants.[2] Many therapeutic compounds isolated from medicinal plants and used as antioxidants, free radical neutralizers, antibacterial agents, or disease prevention supplements.[3] Free radicals originated exogenously from UV light (radiation) or infectious microorganism,[4] or endogenously from metabolic pathways, eventually damaging cells and causing disease progression.[5] Three cultivars of apples (scientific name, Malus domestica), that are widely produced and consumed worldwide are Red Delicious, Golden Delicious, and Granny Smith apples. They are distinguished by their high levels of dietary fiber, vitamins, minerals, and antioxidants.[6-8] Some seeds of apples are usually reserved for cultivation, however, most of them thrown away as a fruit waste. To deal with this waste product, recent studies have focused on detecting phytochemicals present in apple seeds, isolating them, and describing their biological activities.[9] Research findings confirmed the beneficial effects of the three apple seeds in disease prevention and management due to their therapeutic phytochemical content, such as flavonoids, coumarins, and essential oils.[10]

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Therefore, this study was conducted to evaluate and compare the phytochemical profile and antioxidant effect of three seed extracts from Iraqi apples, "Red Delicious, Golden Delicious, and Granny Smith."

Materials and Methods

Source of plant materials
The Red Delicious, Golden Delicious, and Granny Smith apples used for the study were purchased from the local farmers in Mosul City.

Preparation of apple seeds
Each apple from the purchased batches was carefully scoured and cut into four fractions by a sharp house blade after being hand-rubbed with tap water and then with distilled water. The isolated seeds were dried in a dark environment at 25°C for two weeks before being pulverized with grinder and ground into a powder. The powder refrigerated in air-free/light-free containers until it was extracted with methanol, chloroform, water, and n-hexane as solvents.[11]

Extraction of the apple seeds
Powdered seed material (25 g) was extracted in Soxhlet with 250 mL of each of the four solvents: water, methanol, chloroform, and n-hexane. The extracts were filtered through Whitman No. 1 filter paper. The resulting solutions were then placed inside the fume hood to allow drying. After the solvents were evaporated, the aqueous, methanol, chloroform, and hexane extracts were stored in clearly labeled, tightly closed amber containers and refrigerated until they were used for phytochemical screening research.[12]

Preliminary phytochemical screening of apple seed extracts
To make the stock solution, each of the 100 mg extracts (including aqueous, methanol, chloroform, and hexane extracts), was dissolved (10 ml of initial solvents). The acquired stock solutions were subjected to preliminary phytochemical screening. The presence of alkaloids,

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saponins, flavonoids, tannins, steroids, terpenoids, coumarins, carbohydrates, anthraquinones, phenols, proteins, and glycosides was detected using standard tests for screening phytochemicals.[13-20]

Determination of the antioxidant capacity of apple seed extracts

The scavenging capacity for free radicals of the various crude extracts from the three apple varieties was evaluated using the protocol described by Blois,[15] with slight modifications. The various extracts at varying concentrations (50, 100 and 150 g/mL, respectively) were placed in separate test tubes. Each test tube was filled with 1 ml of 2,2-diphenyl-1-picrylhydrazyl solution (DPPH; 0.1 mM), dissolved in methanol, and rapidly shaken. After injecting the DPPH solution, each test tube was gently shaken and left to stand for 45 minutes at 27°C in a dark environment. Blanks and positive controls were created in the same manner but without any extract. In exactly the same proportions, ascorbic acid was employed as a standard. Using UV spectroscopy at a wavelength of 517nm, the test samples' absorbance was calculated.[21] Each experiment was replicated three times in this study. The inhibition percentage of the radical scavenging activity of the test crude extracts was calculated using the following equation:

$$\%\text{Inhibition} = \frac{(Acontrol - Asample)}{Acontrol} * 100$$

Where $A_{control}$ is the absorbance value of the control; A_{sample} is the absorbance value of the sample.

Statistical analysis

All the experiments were replicated three times, and the values are presented as mean±standard error of the mean. The IBM SPSS (V26, USA) was used for the statistical analysis.

Results and Discussion

The yield of crude apple seed extracts

The three apple varieties were extracted with water, methanol, chloroform, and hexane using a Soxhlet extractor. Once the extraction was completed, the solvents were evaporated using a rotary evaporator, yielding semisolid bulk crude extracts. The crude extracts were dissolved in the extracted solvents.

Phytochemical contents of apple seed extracts

The results of the phytochemical screening of the three varieties of apple seed extracts are presented in Table 1. It was observed that the seed extracts of the three apple varieties contained alkaloids, flavonoids, saponins, and tannins. However, when the crude extracts were tested for glycosides and anthraquinones, none of them changed color. All the aqueous crude extracts were positive for carbohydrates, alkaloids, phenols, protein, and saponins. A positive result was obtained for coumarin only in the Golden apple seed extract and negative in the Red and Granny Smith apple seed extracts. Only Golden apple seed extract yielded a positive tannin result, while Red and Granny Smith apple seed extracts yielded a negative result. Terpenoids were found in Golden and Granny Smith apples, but not in Red apple seed extract. Protein levels were high in Golden and Granny Smith apples, but low in Red apple seed extract. All three apple varieties' methanol crude extracts yielded positive results for alkaloids, flavonoids, coumarins, tannins, phenols, and carbohydrates. Terpenoids were found to be present in Red apple seed extract but not in Granny Smith or Golden apple seed extract. Protein levels were found to be high in Red and Granny Smith apple seed extracts, but low in Golden apple seed extract. Saponins in the Red apple seed extract were negative, while saponins in the Golden apple seed extract were positive. Alkaloids, coumarins, phenols, steroids, protein, and terpenoids were found in chloroform extracts of the three apple seed extracts. The only Red apple seed extract was positive for flavonoids, while Golden and Granny Smith apple seed extracts were negative. All extracts yielded negative results for tannin and carbohydrates. The results for saponins in Golden and Granny Smith apple seed extracts were positive, but negative in Red apple seed extracts. Hexane crude extracts of all apple varieties yielded positive results for carbohydrates, alkaloids, and saponins. Protein levels were found to be high in Red and Granny Smith apple seed extracts. All extracts yielded negative results for flavonoids, coumarins, tannins, phenols, and terpenoids.

Antioxidant capacity of apple seed extracts

As the concentration of organic extracts from Red Delicious, Golden Delicious, and Granny Smith apple seed extracts increased, so did the absorbance, as shown in Figures 1-3, respectively. The antioxidant capacity of Red Delicious apple seed extract was in the order methanol > chloroform > hexane > aqueous extract as measured by the DPPH assay.

Table 1: Biochemical examination of Red Delicious, Golden Delicious, and Granny Smith apple seed crude extracts in water, methanol, chloroform, and hexane.

Extract	Red Delicious apple				Golden Delicious apple				Granny Smith apple			
	Aqueous	Methanol	Chloroform	Hexane	Aqueous	Methanol	Chloroform	Hexane	Aqueous	Methanol	Chloroform	Hexane
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+	-	-	+	+	-	-
Coumarins	-	+	+	-	-	+	+	-	-	+	+	-
Tannins	-	+	-	-	+	+	-	-	-	+	-	-
Phenols	+	+	+	-	+	+	+	-	+	+	+	-
Terpenoids	-	+	+	-	+	-	+	-	+	-	+	-
Carbohydrates	+	+	-	+	+	+	-	+	+	+	-	+
Anthraquinones	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	+	-	-	+	+	+	+	+	+	-	+	+
Proteins	-	+	+	+	+	-	+	-	+	+	+	+

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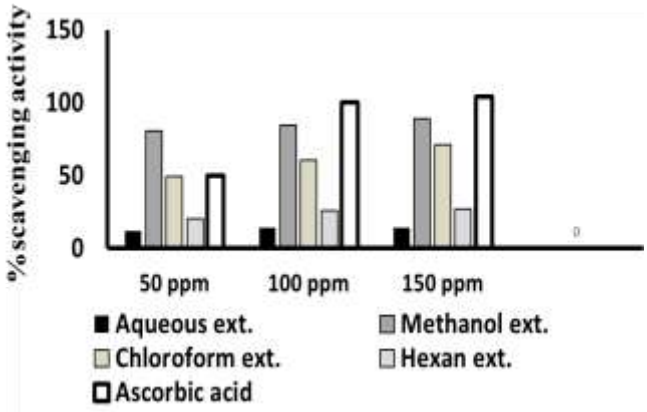

Figure 1: Antioxidant potential of several Red Delicious apple seed crude extracts.

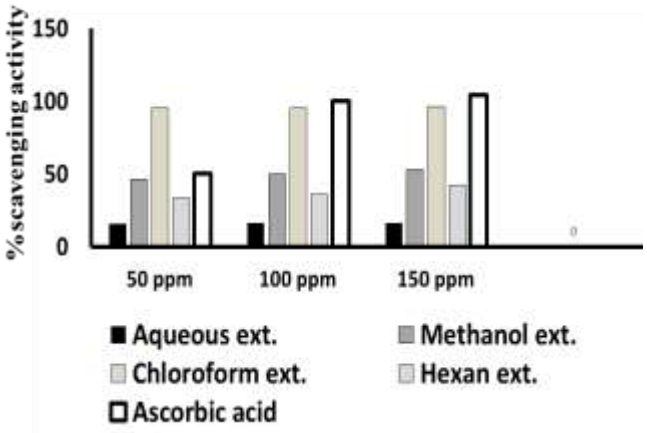

Figure 2: Antioxidant potential of several Golden Delicious apple seed crude extracts.

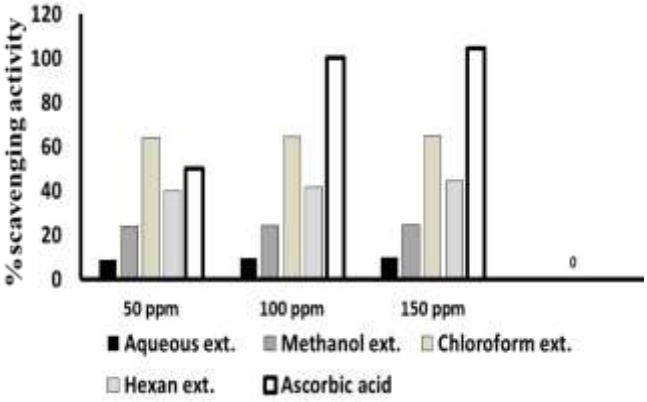

Figure 3: Antioxidant potential of several Granny Smith apple seed crude extracts.

The antioxidant activity of Golden Delicious apple seed extract was in the sequence chloroform > methanol > hexane > aqueous extract, whereas that of Granny Smith apple seed extract was in the sequence chloroform > hexane > methanol > aqueous extract (Figures 1-3). Plant samples contain phytochemical compounds, which are known to be physiologically active substances that are responsible for actions such as antioxidants, antibacterial, antifungal, and other properties, as well as anti-cancer properties.[22,23] Through various biological mechanisms, all secondary metabolites have antioxidant properties. Most secondary metabolites were isolated and named from plant crude extracts using polar solvents.[24] According to the phytochemical screening of methanol, chloroform, hexane, water, and crude extracts from "Red Delicious, Golden Delicious, and Granny Smith apple seed" extracts in this study, the extracts contained flavonoids, alkaloids, tannins, and Saponine (Table 1). Flavonoids, alkaloids, coumarins, tannins, and phenols were discovered in the phytochemical screening of methanol extracts. Saponins were also discovered in other apple extracts from the three varieties. The most potent bioactive components, alkaloids, and flavonoids were found in polar water and methanol extracts. The antioxidant activity can be attributed to specific bioactive components found in crude extracts from the three apple varieties. Several studies have been published on flavonoids with high potential bioactivities such as anti-inflammatory, antioxidant, antibacterial, anti-angiogenic, anti-allergic responses, and ant-cancer.[25-28] Saponins are another bioactive component that has antioxidant activity and helps in plant defense.[29,30] Tannins and their derivatives are phenolic substances that act as free radical scavengers and antioxidants.[29-34] The antioxidant activity of four different crude extracts from Red Delicious (Figure 1), Golden Delicious (Figure 2), and Granny Smith (Figure 3) apple seed extracts at concentrations of 50, 100, and 150 g/mL was evaluated using the free radical scavenging activity (DPPH) assay. The action of antioxidants to generate free radicals, which react with the antioxidants during the DPPH assay, is the basis of antioxidant capacity. A-diphenyl-b-picrylhydrazyl (DPPH) is changed into a-diphenyl-b-picrylhydrazine during the free radical reaction. The rate of color change slows over time, indicating the antioxidant's ability to scavenge. Flavonoids, saponins, tannins, phenolics, and fragrant compounds are found in the seeds of the three apple varieties. All of these bioactive chemicals were able to produce a color after reaction with DPPH because of their hydrogen-donating abilities.[29-33] According to the present study, the four crude extracts from the seed of the three apple varieties have hydrogen-donating properties and will act as antioxidants. The potentials for scavenging free radicals of Red Delicious apple seed extract were methanol > chloroform > hexane > aqueous extract, chloroform > methanol > hexane > aqueous extract, chloroform > hexane > methanol > aqueous extract, and chloroform > hexane > methanol > aqueous extract for Golden Delicious apple seed extracts, and chloroform > hexane > methanol > aqueous extract for Granny Smith apple seed extracts. All crude extracts, on the other hand, exhibited no similar patterns. The variation in activity reported in all crude extract samples could be attributed to extraction procedures, sample processing, or drying. Certain active volatile chemicals in dry samples may be removed or vaporized during sample processing. This could explain why some extracts have lower antioxidant activity than others.

Conclusion

The findings of this study revealed that the antioxidant capacity of Red Delicious, Golden Delicious, and Granny Smith apple seed extracts is dependent on phytochemical constituents such as flavonoids, alkaloids, tannins, and steroids, according to phytochemical screening. The crude extracts from these plants could be used to create new antioxidant agents. More research is required to isolate and identify bioactive constituents found in extracts that could be used in medical applications.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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