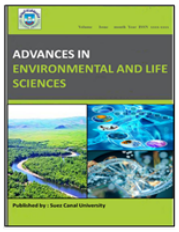
Advances in Environmental and Life Sciences 5(1) (2024) 10-17

Contents lists available at Egyptian Knowledge Bank

Advances in Environmental and Life Sciences

journal homepage: https://aels.journals.ekb.eg



# Phytochemical Screening of Some Nutraceutical Fruits and Leaves Extracts *

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**Abstract**

Bioactive compounds isolated from herbal extracts, dietary nutrients, fruits, and diets may confer physiological advantages that can enhance health and mitigate the risk of chronic disorders. Recent studies have prioritized the exploration of the potential of medicinal plants in modifying chemical pharmaceutical adverse effects through their therapeutic or preventative properties. Consequently, the purpose of this study was to investigate the phytochemical constituents of fruits and leaves extracts of strawberries, and long mulberry. The dried fruits and leaves of berries underwent a triple extraction process using 70% ethanol. The filtration process was conducted on each mixture utilizing filter paper, followed by evaporation in a rotary evaporator. Various qualitative tests were employed to determine the phytochemical constituents present in berries, such as steroids, alkaloids, amino acids, proteins, reducing sugar, glycosides, tannins, flavonoid, and saponins. Furthermore, the quantification of total phenols, total flavonoids, total antioxidants, and phenolic acids was conducted. The extracts derived from the leaves of strawberry, and long mulberry exhibit a higher concentration of total phenols, total flavonoids, and total antioxidants in comparison to their respective fruits.

## 1. Introduction

The term nutraceutical refers to compounds that are isolated from herbal extracts, dietary nutrients, specific fruits, and diets. These herbal compounds probably have physiological benefits that may improve health and protect against chronic diseases **[1, 4].** Recent studies focused on using some medicinal plants for therapeutic or protective purposes against diseases to prevent the side effects of chemical drugs **[2].** Moreover, medicinal plants exhibit anti-diabetic, -anti-oxidative, anti-carcinogenic, and anti-inflammatory characteristics **[3, 4]. The** consumption of berries, specifically strawberry, raspberry, blackberry, blueberry, cranberry, and mulberry, can confer health advantages due to their rich nutritional composition, which includes fatty acids, dietary fiber, minerals, vitamins, and also a diverse array of polyphenolic phytochemicals (phenolic acids, flavonoids, tannins, and lignans) **[5].** The Morus genus of the family Moraceae includes two species of mulberry, namely, blackberry (Morus nigra) and white berry (Mours alba). The black mulberry fruits have been found to possess a higher total phenolics content (TPC), total flavonoid content (TFC), and anthocyanins in comparison to the white mulberry fruits **[6] .** Strawberry (Fragaria ananassa), a member of the Ericaceae family, has been found to have a significant content of bioactive compounds and antioxidants **[7].** The health benefits of strawberries are attributed to their high content of phenolics, such as polyphenols and flavonoids, as well

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doi 10.21608/AELS.2023.233258.1038

Received: 31 August 2023, Revised:23 September 2023
Accepted:1 October 2023, Published: 1 January 2024

as micronutrients like minerals, folate, and vitamin C **[8, 9]. Ant**hocyanins are the most abundant polyphenolic compound present in strawberries. The bioactive compounds found in strawberries have been shown to exhibit significant biological actions in studies conducted in vitro and in vivo, thereby offering potential prevention or treatment against various chronic diseases **[10, 11]. These** compounds have been found to possess potent anti-oxidative, anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-metabolic syndrome, anti-obesity, and anti-microbial effects **[12].**

In traditional medicine, leaf extracts of the above-mentioned fruits have been used in both the treatment and protection of diabetes, inflammation of the urinary tract, and colds **[13]. Mu**lberry leaves are rich in not only organic acids and essential macro- and micronutrients but also in protein, ascorbic acid, and minerals, especially calcium and potassium, which are the most prevalent elements **[14]. St**rawberry leaves contain multiple polyphenolic compounds, which include flavonoids, tannins, and essential oil, that have an antioxidant effect **[15] . S**trawberry leaves have been used as an appetizer for treating diabetes, hypertension, anemia, and hypercholesterolemia **[16].**

The current study was performed to assess the phytochemical composition of fruit and leaf extracts from two types of berries (long mulberry and strawberry) and also to determine their TPC, TFC, and total antioxidant.

## 2. Materials and Methods

### *2.1. Plant collection*

Herein, the fruits and leaves of berries were collected from a farm in Ismailia, Egypt. Then washed with tap water 2-3 times and kept in a dry place to evaporate the water content. After complete drying in air, samples were crushed with a mechanical blender to get fine powder for each sample **[17].**

### *2.2. Plant Extraction*

The dried fruits and leaves of berries were extracted with 70% ethanol three repeated times. Each mixture was filtered with filter paper and evaporated in a rotary evaporator. The obtained ethanol extract from berry fruits and leaves was kept at -20°C until subsequent use **[18]. T**hen the dried extract was analyzed.

### *2.3. Qualitative phytochemical screening*

The phytochemical constituents of berries were determined by different qualitative tests, including steroids, alkaloids, amino acids, proteins, reducing sugar, glycosides, tannins, flavonoid, and saponins **[18].**

### *2.4. Detection of steroids*

For steroid detection, 10 mg of extract was dissolved in 2 ml of chloroform, followed by adding sulfuric acid to the test tube's side according to Libermann Buchard's reaction. After a few minutes of shaking the test tube, the identification of steroids within the extract was demonstrated by forming a reddish color in the chloroform layer and greenish-yellow fluorescence in the lower layer **[19].**

### *2.5. Detection of alkaloids*

For alkaloid detection, 2 mg of the extract was mixed with 2 mL of picric acid. The formation of an orange color is indicative of the presence of alkaloids **[20].**

### *2.6. Detection of amino acids by ninhydrin test*

For amino acid detection, 1 mL of an alcoholic solution containing 0.1% ninhydrin was introduced into the extract. The formation of purple color is indicative of the presence of amino acids **[21].**

### *2.7. Detection of proteins by Xanthoprotein test*

For protein detection, 2 ml of water was introduced to a small quantity of residue, followed by adding 0.5 ml of concentrated nitric acid and subsequent mixing. The confirmation of protein existence was established by forming a white or yellow precipitate **[22].**

### *2.8. Detection of reducing sugar by Fehling's reagent*

For reducing sugar detection, 2 mg of extract was combined with 0.5 mL of Fehling's reagent, which consisted of a mixture of Fehling's solution A and B that had been prepared prior to usage. After adding 2 mL of sodium hydroxide solution to the mixture, the mixture was subjected to heating in a water bath at 100º C for a duration of 10 minutes. The reddish-brown precipitate observed in the sample indicates the presence of reducing sugars **[23].**

### *2.9. Detection of glycosides by Fehling's reagent*

For glycoside detection, the solution procured through Fehling's assay was used. After adding a few drops of diluted HCl to the clear solution, the mixture was boiled for 5 minutes to hydrolyze the glycosides. Fehling's reagent was re-added to detect if there was any additional reduction that could indicate the existence of glycosides **[23].**

### *2.10. Detection of tannins*

For tannins detection, 10 ml of distilled water was added into a beaker containing 5 mg of the residue obtained from the extract. Subsequently, the mixture was subjected to boiling for 5 minutes, followed by adding drops of ferric chloride solution ($FeCl_3$). The formation of green color was indicative of tannins presence **[19].**

### *2.11. Detection of flavonoids*

For flavonoid detection, 1 mg of the extract was added to 2 drops of dilute sodium hydroxide solution. Flavonoids were detected by the development of a strong yellow color which subsequently turned colorless upon adding a few drops of diluted acid **[19].**

### *2.12. Detection of saponins by Foam test*

For saponin detection, 10 mg of the extract was added to a test tube that contained a small amount of sodium bicarbonate and distilled water and was then strongly shaken. The appearance of foam was indicative of saponins presence **[19].**

### *2.13. Determination of total phenolic content (TPC)*

The Folin-Ciocalteu method, as described by Zilic, et al **[24]**was utilized to determine the TPC of the extracts. After adding 100 $\mu$L of the extract into a test tube, the resulting volume was made up to 3.5 mL using distilled water. The mixture was subjected to oxidation by adding 250 $\mu$L of Folin-Ciocalteau reagent. After a period of 5 minutes, the mixture was neutralized using a 20% aqueous solution of sodium carbonate ($Na_2CO_3$) with a volume of 1.25 ml. Subsequently, the absorbance was measured at a 725 nm wavelength relative to the solvent blank after a duration of 40 minutes. The quantification of TPC was conducted utilizing a calibration curve that was prepared through gallic acid. The results were reported as micrograms of gallic acid equivalent (mg GAE)/gram of the sample **(Fig.1).**

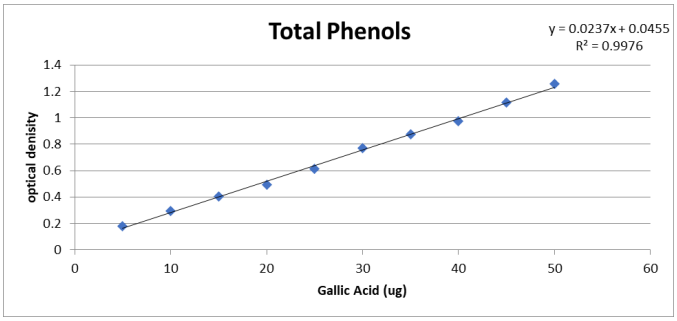


Figure 1: Calibration curve constructed from knownconcentrations of gallic acid

### *2.14. Determination of total flavonoid content (TFC)*

The TFC quantification was conducted through the utilization of the aluminum chloride ($AlCl_3$) colorimetric assay, as per the methodology outlined by Zilic, et al [24]. First, 100 $\mu$L of the extract was mixed with 300 $\mu$L of a solution containing 5% sodium nitrite ($NaNO_2$). Following 6 minutes, an amount of 300 $\mu$L of a solution containing 10% $AlCl_3$ was introduced, and the overall volume was modified to 2.5 mL by adding distilled water. Subsequently, following 7 minutes, a volume of 1.5 mL of 1 M NaOH was added into the solution, which was then subjected to centrifugation at 5000 g for 10 min. The supernatant absorbance

was measured at a 510 nm wavelength, with the solvent blank serving as the reference. The quantification of the TFC was conducted using a calibration curve that was prepared using catechin. The obtained results were reported as milligrams of catechin equivalent (mg CE)/gram of the sample. Additional dilution was performed in cases where the measured absorbance value exceeded the linear range of the established standard curve **(Fig.2).**

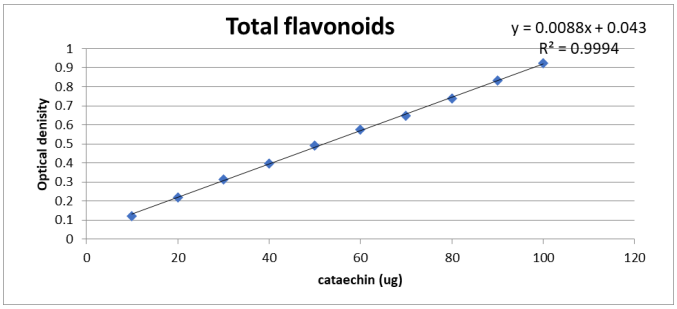


Figure 2: Calibration curve constructed from known concertation of catechin

### *2.15. Determination of total antioxidant activity (DPPH scavenging activity)*

The stable DPPH* was employed to evaluate the extract free radical scavenging capacity, as described by Hwang and Thi **[25]. T**he DPPH* was present in a final concentration of 200 $\mu$M, and the final volume of the reaction was 3.0 ml. Measuring the absorbance was conducted at a 517 nm wavelength, utilizing pure methanol as a blank, subsequent to a 60-minute incubation period under conditions of darkness. The calculation of the percentage inhibition of the DPPH free radical was performed as follows:

Inhibition (%) = 100 × [(A control - A sample)/A control]

Where:

A control refers to the control reaction absorbance (which comprises all utilized reagents except for the test compound). A sample refers to the test compound absorbance. Trolox was utilized to prepare the standard curve. The outcomes were presented in units of $\mu$g Trolox equivalents (TE)/g of the sample **(Fig. 3)**.

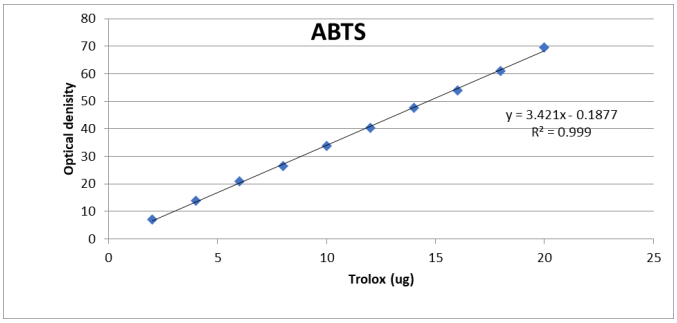


Figure 3: Calibration curve constructed from known concentrations of Trolox

### *2.16. Determination of phenolic acids profile*

The HPLC analysis of the extracts was conducted at the National Research Center (NRC) located in Dokki, Cairo. The procedure was conducted following the methodology of Kim, et al [26] utilizing an Agilent Technologies 1100 series liquid chromatograph that was equipped with an autosampler and a diode-array detector. The analytical column utilized in the experiment was an Eclipse XDB-C18 (150 X 4.6 $\mu$m; 5 $\mu$m) accompanied by a C18 guard column (Phenomenex, Torrance, CA). The mobile phase comprised acetonitrile as solvent A and a mixture of 2% acetic acid in water (v/v) as solvent B. The experiment was conducted with a consistent flow rate of 0.8 ml/min over a period of 60 minutes. The gradient program employed included four stages as follows: 100% to 85% B in 30 min, 85% to 50% B in 20 min, 50% to 0% B in 5 min, and 0% to 100% B in 5 min. The injection volume utilized was 50 $\mu$l, and prior to injection, all specimens underwent filtration using a 0.45 $\mu$m Acrodisc syringe filter (Gelman Laboratory, MI). The benzoic acid and cinnamic acid derivatives were monitored concurrently at 280 and 320 nm, respectively, while the flavonoids were monitored at 360 nm. The identification of peaks was conducted through the congruence of their retention times and UV spectra, which were subsequently compared to the established standards **(Fig. 4)**.

## 3. Results and discussion

### *3.1. Qualitative phytochemical screening*

The data shown in Table. 1 demonstrate the presence of many phytochemicals in all exam-

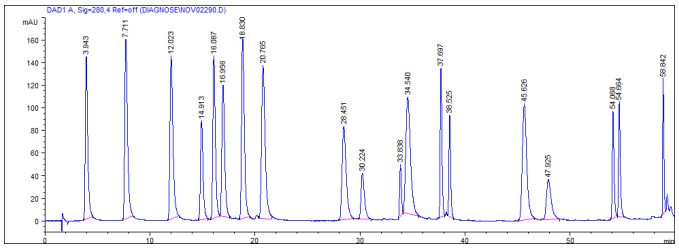

Figure 4: HPLC peaks of the standards

ined berries, including alkaloids, steroids, amino acids, proteins, reducing sugars, glycosides, tannins, flavonoids, and saponins at high concentrations in several types of berries. So, the present findings indicate that the health-promoting benefits of fruits and leaves are mostly attributable to their phytochemical contents, particularly their phenolic compounds **[27].**

The main phytochemical families that are present in long mulberry fruit (LMF) are alkaloids, steroids, proteins, amino acids, reducing sugar, flavonoids, glycosides, tannins, and saponins are not present in OMF. In contrast, the main phytochemical families that are present in long mulberry leaves (LML) are steroids, proteins, glycosides, tannins, and flavonoids, while phytochemical families: alkaloids, amino acids, reducing sugar, and saponins are not present in LML.

The main phytochemical families that are present in strawberry fruits (SF) are alkaloids, amino acids, proteins, reducing sugar, glycosides, and flavonoids, although the phytochemical families that are not present in SF: steroids and tannins. Whereas the phytochemical families present in strawberry leaves (SL) are amino acids, proteins, reducing sugar, glycosides, tannins, and flavonoids but phytochemical families are not present in SL: steroids, alkaloids, and saponins.

### *3.2. Total phenolic contents (TPCs):*

The TPCs of LML, SL, LMF, and SF were assessed with the Folin-Ciocalteu method and were reported as mg GAE/g of the sample. The TPCs values of LML, SL, LMF, and SF are 77.593, 10.040, 15.594, and 4.821 mg GAE/g, respectively (Table 2).

Table 1: Phytochemical analysis of berries

| Test | LMF | LML | SF | SL |
|---|---|---|---|---|
| **Steroids** | + | + | - | - |
| **Alkaloids** | + | - | + | - |
| **Amino acids** | + | - | + | + |
| **Proteins** | + | + | + | + |
| **Reducing sugar** | + | - | + | + |
| **Glycosides** | + | + | + | + |
| **Tannins** | + | + | - | + |
| **Flavonoids** | + | + | + | + |
| **saponins** | - | - | - | - |

LMF:long mulberry fruit, LML: long mulberry leaves, SF: strawberry fruit, SL:strawberry leaves. (+) representas present, (-) represent as absent.

### *3.3. Total flavonoid contents (TFCs)*

Flavonoids, a wide group of phenolic compounds with health-promoting properties, are among the most important components of edible wild fruits. The TFCs of LML, SL, LMF, and SF were determined by $AlCl_3$ colorimetric assay and were reported as mg CE/g of the sample. The TFCs values of LML, SL, LMF, and SF are 49.925, 6.314, 5.790, and 3.483 mg CE/g, respectively (Table 2).

### *3.4. Antioxidant activity (DPPH scavenging activity)*

Antioxidant activity of LML, SL, LMF, and SF was determined using the stable DPPH and was reported as TE/g of the sample. DPPH scavenging activity values of LML, SL, LMF, and SF are 214.035, 22.142, 13.645, and 3.558 mg TE/g, respectively (Table 2).

TPC and TFC are often utilized to designate the differences in phenolic contents among different species. Table. 1 summarizes the used TPC, TFC, and antioxidant capacity of the examined berries. The TPC and TFC value of berries were found to be subject to variations due to factors such as the type of extracting solvent, cultivation environment, and growing seasons **[28, 29].** Mulberry fruit has numerous physiological functions that aid in the protection against human disorders, including cancer, arteriosclerosis, as well as brain and cardiovascular disorders **[30].** **P**olyphonic compounds function

as antioxidants through the process of scavenging free radicals or reducing their formation **[31].** Numerous assays have been devised to measure antioxidant capacity; however, the DPPH assay is characterized by its simplicity, affordability, and speed **[32].** The methodologies employed to determine the antioxidant capacity principles exhibit significant variability depending on the type of radical produced or the duration of the reaction. The DPPH assay exhibits significant variations in its reactivity towards antioxidant activity. Consequently, it is strongly advised to employ diverse techniques for assessing the antioxidant properties of food substances in order to acquire a comprehensive understanding [33]. Previous research has indicated a significant correlation between antioxidant activity and both TPC and TFC in various plant species **[34].**

The observed fluctuations in the phenolic content of mulberry leaves can be attributed to the variations in the extraction methodologies and analytical approaches utilized in each investigation. Furthermore, [35] have suggested that the levels of phenolic compounds present in leaves are subject to fluctuations based on a range of environmental factors, which include dryness, temperature fluctuations, pollution, and exposure to UV radiation.

Table 2: Total phenols, Total flavonoids, and antioxidant activity (DPPH) of berries

| Sample | Total phenols (mg GAE/g) | Total flavonoids (mg CE/g) | DPPH (mg TE/g) |
|---|---|---|---|
| **LML** | 77.593 | 49.92593 | 214.035 |
| **SL** | 10.040 | 6.314 | 22.142 |
| **LMF** | 15.594 | 5.790191 | 13.645 |
| **SF** | 4.821 | 3.483796 | 3.558 |

LMF:long mulberry fruit, LML: long mulberry leaves, SF: strawberry fruit, SL:strawberry leaves. GAE= gallic acid equivalent, CE= catechineequivalent, DPPH=diphenylpicryl hydrazine, TE= trolox equivalent

Table 3: Phenolic profile of SL, SF, LMF, and LML (expressed as mg phenol/100g extract)

| Compound | RT | SL | SF | LMF | LML |
|---|---|---|---|---|---|
| **Gallic** | 3.9 | ND | ND | ND | ND |
| **Protocatechuic** | 7.7 | ND | ND | 3.78 | ND |
| **p - hydroxybenzoic** | 12.0 | 164.06 | 6.61 | 3.32 | 6.754 |
| **Gentisic** | 11.9 | 15.14 | 10.45 | ND | 13.353 |
| **Cateachin** | 14.9 | 32.78 | ND | ND | ND |
| **Chlorogenic** | 16.1 | 20.86 | 182.40 | 16.67 | 28.165 |
| **Caffeic** | 16.9 | 4.04 | 2.29 | 1.23 | 6.431 |
| **Syringic** | 18.8 | 7.85 | ND | 1.55 | ND |
| **Vanillic** | 20.8 | 17.75 | 11.58 | 0.98 | 0.568 |
| **Ferulic** | 28.5 | 3.28 | 2.90 | ND | 0.389 |
| **Sinapic** | 30.2 | 10.74 | 21.29 | ND | ND |
| **p -coumaric** | 34.5 | 6.62 | 1.21 | 0.95 | 6.230 |
| **Rutin** | 33.8 | 6.80 | 31.31 | 3.60 | 12.637 |
| **Apigenin-7-glucoside** | 37.7 | 37.74 | 0.89 | 3.92 | 5.597 |
| **Rosmarinic** | 38.5 | 5.40 | 24.18 | 0.35 | 6.582 |
| **Cinnamic** | 45.6 | ND | 0.77 | 2.23 | ND |
| **Qurecetin** | 47.9 | ND | ND | ND | ND |
| **Apigenin** | 54.1 | ND | ND | 1.57 | ND |
| **Kaempferol** | 54.7 | ND | ND | ND | ND |
| **Chrysin** | 58.8 | ND | ND | 0.29 | ND |

RT= retention time, ND= not detected

## 4. Phenolic acid profile

The type and quantity of some individual polyphenols in SL, SF, LMF, and LML are shown in Table. 3. P-hydroxybenzoic acid and apigenin-7-glucoside were found to be the major polyphenol in the SL at concentrations 164.06 and 37.74 mg/100g, respectively. At the same time, ferulic acid and caffeic acid are phenolic acids and were found to be the minor polyphenols in the extract.

In SF, chlorogenic acid and rutin were found to be the major polyphenol at concentrations 182.40 and 31.31 mg/100g, respectively. At the same time, apigenin-7-glucoside and cinnamic acid were found to be the minor polyphenols in the extract.

In LMF, chlorogenic acid is phenolic acid was found to be the major polyphenol in the extract at a concentration of 16.67 mg/100g. However, rosmarinic acid and p-coumaric acid were the minor

polyphenols in the extract.

In LML, chlorogenic acid and gentisic were found to be the major polyphenol at concentrations of 28.165 and 13.353 mg/100g, respectively. At the same time, vanillic acid and ferulic acid are phenolic acids and were found to be the minor polyphenols in the extract.

## 5. Conclusion

Phytochemical screening is an important primary test used to detect the presence of different phenolic compounds in leaves and fruit extracts, which may have significant importance in the medication of several diseases. The present results demonstrated significant differences in TPC and TFC and the total antioxidant capacity of leaves and fruits of tested berries. Leaves of berries have a higher concentration of TPC, TFC, and antioxidant capacity than fruits of berries.

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