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Study regarding the development of an inovative mixture of plants with health benefits

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Abstract

A mixture of plants can be considered a food or part of a food and provides medical or health benefits like the prevention and treatment of disease. Spices and herbs as well as fruits are rich sources of powerful antioxidants and bioactive compounds. The main objective of the study was the comparative evaluation of phenolic compounds of different mixtures of plants methanolic extracts and also the evaluation of the antioxidant activity of these plant extracts using specific method. In this study three plants and fruits (apple, orange and mint) were mixed (in dried form) in different proportion and the mixtures' content in polyphenols and their antioxidant capacity were determined. The total phenolic content in plant extracts was quantified using the Folin-Ciocalteu method and the antioxidant activity of plants was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The total phenolic content was between 27.74 and 24,10 mg GAE/g, while the antioxidant capacity ranged between 31.71% and 29.13% for dried spices. The present research work, demonstrates the nutritive value of selected plants, revealing their potential as natural antioxidants in food industry.

Keywords: apple, antioxidant activity, mint, orange, total polyphenols, vitamin C

1. Introduction

Nowadays, plant food, especially vegetables and fruits, have been given great attention due to their health benefits. In the past decade, numbers of studies have found that they are a great source of natural antioxidants [9] and they are important healthy diet components that can help prevent multifarious chronic diseases [4].

Fruits and vegetables contain a variety of substances, e.g., vitamins, minerals, and phytochemicals that may be beneficial for cognitive function, either alone or in combination. However, from a public health perspective, it is easier to recommend whole foods instead of single nutritional components. In addition, isolated pure compounds either lose their bioactivity or may not behave the same way as the compounds in whole foods [2].

Apples are one of the most commonly consumed fruits in the world. Apples are an excellent source of phenolic compounds, these compounds being responsible for most of the antioxidant activity of the fruit [8]. The famous sentence: "*An apple a day keeps the doctor away*!" is what is highly recommended and heavily advertised nowadays to the general public to stay fit and healthy. This claim is due to the high nutraceutical values of the apple's compounds and to the large abundance and accessibility of this fruit in the market.Also due to postharvest storage

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technology, fruit shelf life can be extended for up to one year, depending on the variety. Although apples are one of the most consumed fruits in the world it is important to note that they are not those with the greatest phenolic content and antioxidant capacity. [3].

Oranges (*Citrus sinensis*) are probably the most recognized and globally accepted type of fruit. Oranges contain many essential nutrients, vitamins, minerals, carotenoids, flavonoids, dietary fiber, phytochemicals and play a major role in overall well-being of humans. The high antioxidant activity of orange juice has been linked with important health benefits such as treating kidney stones, controlling the levels of cholesterol and high blood pressure, minimizing the risk of stroke or cancer [6].

Mentha piperita L., member of the large mint family *Lamiaceae*, is a fast-growing, perennial herb which can reach up to 1.5m height (in favorable conditions). *M. piperita* is an extremely variable species with a widespread distribution in Europe. Mint herb is used in medicine, cosmetics and food industry, therefore, this specie is widely grown around the world. The leaves are used for relief of minor sore throat and minor mouth or throat irritation. It is also used in treatments for minor aches and sprains, and in nasal decongestants in addition to its antiparasitic, carminative, antiseptic and stimulant properties [8].

2.Materials and Methods

The analyzed samples (apple, orange and mint) were purchased from local supermarkets (Cluj-Napoca, Romania) and were mixed in dried form in different proportion. First mixture contained 50% apple, 48% orange and 2% mint and for the second mixture 60% apple, 36% orange and 4% mint were used. The mixtures were investigated in respect with the total polyphenol content, antioxidant capacity and ascorbic acid.

Preparation of extracts

The methanolic extracts were obtained according to the method described by Muresan *et al*., 2012 [8]. For sample extraction, 1g of powdered material was extracted with 10 ml of methanol. The extract was separated and the residual tissue was re-extracted until the extraction solvents became colorless. The filtrates were combined in a total extract, which was dried by vacuum rotary evaporator at 40°C. The dried residues were redisolved in methanol and stored in a freezer at -20°C until analyzed.

Total phenolic content

The determination of the total phenolics content was performed using the Folin-Ciocalteu reagent, according to Muresan *et al.* (2012) [8] and Kodama *et al.* (2010) [7] with some modifications. A 0.25 mL of the methanolic extract obtained above were mixed with 0.12 mL of the Folin-Ciocalteu reagent and 1.8 mL of distilled water. After 5 minutes at room temperature, 0.34 mL of a sodium carbonate (Na_2CO_3) solution 7.5% were added and the mixture placed at room temperature for 2 hours. The absorbance was measured at 750 nm on a Shimadzu UV-1700 PharmaSpec spectrophotometer. A calibration curve was performed using different concentrations of standard gallic acid solutions (r^2 = 0.9997) and the concentration of TPC was expressed as mg GAE/g dried material.

Determination of 2,2-diphenylpicrylhydrazil radical scavenging capacity (DPPH)

Scavenging activities of the extracts on the stable free radical DPPH were assayed using the method adapted after Anesini *et al*. 2008 [1]. A volume of 0.1 mL of an methanolic extract were mixed with 0.9 mL distilled water and 3.9 mL methanolic DPPH solution. After 30 minutes incubation in darkness, the absorbance of each sample was measured at 515 nm against a blank of methanol. The percentage of DPPH was calculated by measuring the absorbance of the sample and applying the following equation:

$$\% \text{ of inhibition} = [1 - (A_s/A_0)] \times 100,$$

where A_s is the absorbance of sample, and A_0 is the absorbance of the DPPH solution.

Determination of ascorbic acid

For determination of ascorbic acid the titrimetric method with potassium iodate was used. Five g of analyzed samples were mixed with a volume of 10 mL hydrochloric acid 2 % and 2,5g quartz sand. After 10 min. this solution was transferred to a 50 mL volumetric flask and completed with hydrochloric acid 2 %. After that, the solution was

filtered. For analysis 10 mL of this filtrate were mixed with 5 mL KI 1% and 1,5 mL starch solution 0,2%. Finally, titration was performed with potassium iodate until a purple-blue coloration appeared.

Calculation: vit C = $V*V_1*0,352/G*V_2*100$

where V= volume of potassium iodate used of titration, V_1= volume of the total extract, G = the quantity of sample, V_2 = volume of extract taken for analyses

3.Results and discussion

Results of the colorimetric analysis of total phenolics together with the free radical scavenging activities and vitamin C content of the analyzed extracts from the two mixtures of plants and fruits are given in Table 1.

Table 1. The content of total phenols, antioxidant activity and ascorbic acid of two mixtures of plants and fruits

Species	Total phenols (mg GAE/g)	DPPH %	Vitamin C mg%
Mixture 1 (50% apple, 48% orange and 2% mint)	27.74±0.707	31.71±0.007	53.2±0.707
Mixture 2 (60% apple, 36% orange and 4% mint)	24.10±1.414	29.13±0.085	68.64±0.000

The highest concentration in phenolic compounds was determined for the first mixture of plants and fruits (50% apple, 48% orange and 2% mint) 27.74±0.707 mg GAE/100g, followed by mixture 2 (60% apple, 36% orange and 4% mint) 24.10±1.414 mg GAE/100g .

The ranking of antioxidant activity values of mixture extracts were found to be the same with the ranking of values of the total phenolic content for the same plant extracts. Thus, first mixture (50% apple, 48% orange and 2% mint) had the greatest antioxidant activity. Antioxidant activity of various this plants and fruits has been studied extensively by many researchers and reported to exert high antioxidant potential [5,10].

Ascorbic acid content in analyzed samples differed significantly with the results of the total phenolic content and antioxidant activity.

4.Conclusion

In this study, was assessed the content in total polyphenols and vitamin C as well as the antioxidant capacity of two mixture of plants and fruits. Our results show that these mixtures are rich in phenolic constituents and demonstrate good antioxidant capacities and the use of them in cooking and food processing may have beneficial effects for human health. A positive and significant correlation existed between antioxidant activity and total phenolics, in some selected plants and frutis, revealing that phenolic compounds were the dominant antioxidant components. The data in this report further enlarge the database of vitamin C contents in fruits and vegetables and will serve as a useful guide in the selection of plants which are rich in vitamin C.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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