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Enhancement and Evaluation of Peppermint *(Mentha Piperita L.)* Beverage

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Abstract: **It is well known that, peppermint is one of the most widely consumed single ingredient herbal teas and other products used as an antiseptic, antibacterial activity, stimulant carminative agent or further used as a flavoring agent in cosmetic and pharmaceutical industries. Also, citrus fruits include lime are an important source of antioxidant compounds and minerals. This study was carried out to assessment the possibility of enhancement lime juice and soda water with peppermint extract as a new beverage with highly functional and organoleptically properties. The results showed that the total phenolic and total flavonoid contents of lime juice and peppermint were 193.02, 53.41 and 360.04, 421.96 mg/100g respectively. The results obtained by (HPLC) for all beverage treatments identified and quantified 13 phenolic and 8 flavonoid compounds. Caffeic, ellagic, aminobenzoic, vanillic and P-Hydrobenzoic are the most important and dominant phenolic acids. Moreover, naringin, quercitrin, rutin and hesperetin had the highest levels of flavonoid compound. Regarding sensory evaluation, the peppermint extract added to lime and soda water by (50:5:45) (v:v:v), was the most widely accepted, followed by (45:5:50) and (40:5:55) compared to the other blends and control. These enhancement beverages can be used in therapeutics due to the incorporated herbal extracts that have a widely accepted medicinal application in prevention and treatment of various disorders. Thus, such fortified beverage has potential application in enhancing health benefits and therapeutic applications**

Keywords: Mentha piperita L., Citrus aurantifolia, **phenolic compounds.**

1. INTRODUCTION

For a long period of time, plants have been a variable source of natural products for maintaining human health, especially in the last decade, with more intensive studies devoted to natural therapies. The World Health Organization has recommended that this should be encouraged, especially in places where access to conventional treatment is not adequate. Studies have shown that many plants have chemical components and biological activities that produce definite physiological actions in the body and, therefore, could be used to treat various ailments. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds [4].

Consumer demand for organic foods has continuously increased because of the perception that they might contain greater amount of beneficial components than their conventionally produced counterparts [15]. Over the past few decades, an increase in the consumption of sugared fruit drinks and soft drinks has been documented, particularly in children and adolescents [6]. Adolescents have higher nutrient requirements compared with other age groups because of their rapid growth and development; furthermore, they may develop unhealthy eating habits that lead to inadequate nutrient intake [31].There are direct links between the high intakes of sugar sweetened beverages and decreased intake of high-fiber foods, increased energy intake, and obesity. Although soft drink intake may not be the primary cause of obesity, it has been identified as one of contributory factors in school children.

This is a concern because obesity in adolescence is a risk for early establishment of chronic diseases such as coronary heart diseases, hypertension, and type 2 diabetes [34]. The consumption of soft drinks with phosphoric acid should be considered as an independent risk factor for hypocalcaemia in postmenopausal women. A strong relationship between the consumption of cola soft drinks and hypocalcaemia [12]. Many herbs contain a variety of phytosterols, phenolic acids,

triterpenes, flavonoids, anthocyanins, saponins and carotenoids, which have been shown to exert cancer chemo-preventive and antioxidant properties.

Peppermint (*Mentha piperita L.*) is one of the most widely consumed single ingredient herbal teas, or tisanes. Peppermint tea, brewed from the plant leaves, and the essential oil of peppermint are used in traditional medicines [10]. *Mentha piperita L.* (Family: *Lamiaceae*) commonly known as peppermint. Peppermint with vernacular names of "nana", a plant is traditionally used as an antiseptic, antibacterial activity, stimulant, carminative agent or it is further used as a flavoring agent in cosmetic and pharmaceutical industries throughout the world [24].

Citrus fruits (Family: *Rutaceae*) are an important source of antioxidants such flavonoids, and other phenolic compounds, and also some essential minerals for human nutrition. Citrus fruits, such as lime (*Citrus aurantifolia*) and lime (*Citrus Lime*) are widely available and regularly consumed as whole fruits or fruit juices and preserved snacks. Among the phenolic compounds, flavanones are the major group found in citrus [14]. Various studies have shown that intake of flavonones is associated with reduced risk of developing coronary heart disease, degenerative diseases and anti-carcinogenicity because of their anti-lipid peroxidation [36].

The objective of this study was to investigate the effect of mixing mint extract, lime extract and carbonated water on the content of an important biological activity of phenolic compounds.

2. MATERIALS AND METHODS

2.1 Plant material and extract preparation:

2.1.1 Mint extracts prepared:

Mint (*Mentha piperita L.*) baladi cultiviar was purchased from the local market. The leaves were separated and washed under tap water and then air-dried. To obtain an aqueous extract (Ex), boiling water (100 ml) was poured over the peppermint leaves (25 g) the mixture was boiled for 10 min and passed through a filter (muslin cloth) to remove the plant particles. The freshly squeezed extract was collected for analysis and using in the preparation beverage.

2.1.2 Preparation of lime juice:

Fresh lime fruits (*Citrus aurantifolia*), at the commercial mature stage was purchased from the local market. Healthy fruits were selected randomly for uniformity of shape and color. The fruits were washed thoroughly with potable water and then air-dried. The lime fruit juice was extracted by cutting the fruit into half and carefully hand-squeezing to obtain the juice. The juice was passed through a strainer to remove pulp and seeds. The freshly squeezed juice was centrifuged at 3000 rpm. For 10 min and the supernatant was diluted. The diluted juice was collected to analysis and using in the preparation beverage.

2.1.3 Carbonated water (Fizzy water):

Soda water or Fizzy water was produced in Schweppes Beverage Company Egypt.

2.1.4 Preparation of the deferential treatments:

The peppermint extract was then blended with sugar at a level of about 13 °Brix [11] and its pH value was adjusted to about 3.0 – 4.0 using lime juice. The beverage was boiled for 5 min to eliminate microorganisms followed by filtration. Then the beverage was carbonated by cold fizzy water. It was first pre-chilled in a refrigerator to decrease the temperature to about 2 – 4°C to facilitate the absorption of CO_2 gas. The treatments of carbonated beverage were then bottled and capped immediately to maintain their gas contents as follows:

(T1): 40:5:55% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T2): 45:5:50% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T3): 50:5:45% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T4): 55:5:40% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T5): 60:5:35% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T6): 65:5:30% (v: v: v) of mint extract, lime juice and fizzy water respectively.

(T7): Control: 100% peppermint beverage.

2.2 Analytical Methods:

2.2.1 Physical and chemical analysis:

Total soluble solids (T.S.S), pH value, moisture content, total acidity, ash, were determined according to [3]**.** Browning was measured (absorbance at 420nm) as mentioned by [25] . Total phenolic compounds were determined using the Folin-Ciocalteu method according to [16]. The flavonoid content was measured by a modified aluminium chloride colorimetric method according to [41]. Fractionation and identification of phenolic compounds were determined by HPLC according to the method of [17]**.**

2.2.2 Determination of antioxidant activity (AOA) by DPPH (2,2-diphenyl-1 picrylhydrazyl) radical scavenging method:

The free radical scavenging capacity of the extracts was noted through the change of optical density of DPPH radicals at 517 nm after 20 min incubation at room temperature. The total free radical scavenging capacity of the methanolic crude extracts was determined spectrophotometrically by measuring the disappearance of DPPH radical at an absorbance of 517 nm. DPPH scavenging activity was calculated by using the equation:

$$\% \text{ inhibition of DPPH} = \frac{\text{Abs control} \_ \text{Abs sample}}{\text{Abs control}} \text{x}100$$

Where: Abs., control is the absorbance of DPPH solution without extracts, Abs.; sample is the absorbance of DPPH with solution extracts [26].

2.3 Sensory evaluation: The organoleptic properties of the peppermint, lime beverages produced from the medicinal plants were evaluated according to [30].

2.4 Statistical analysis: The obtained data were statistically analyzed according to ANOVA procedure of the statistical package for the social sciences (SPSS) [32].

3. RESULTS AND DISCUSSION

3.1 Chemical composition of raw materials:

The physical and chemical properties of fresh lime and peppermint are shown in Table (1). Results indicated that fresh lime juice contains higher values for moisture, total acidity and ascorbic acid than those of peppermint. Also, the obtained results in Table (1) showed that ash of peppermint was higher values than those of the lime. The quantitative estimation of the phytochemical constituents of peppermint and lime shows that the medicinal herbs are rich in total phenols, total flavonoids according to the data shown in Table (1). The content of total phenolic (as Gallic acid) and total flavonoid (as Quercetine) in peppermint leaves were 360.04±0.285 and 421.96±0.25 mg/100gm respectively, while they recorded in lime juice193.02± 0.183 and 53.41±0.17 mg/100gm respectively. The results in the same table show that the content of total phenolics and total flavonoids in peppermint extract and lime extract were 90.01±0.123 - 105.49±0.130 mg/100ml and 77.21± 0.089 - 21.36±0.076 mg/100ml respectively. The total phenolic and flavonoid contents of lime juice *Citrus aurantifolia* (Common lime) and *Citrus microcarpa* (musk lime) were reported as 211.70 and 105.0 mg /100gm (as gallic acid) respectively, and 10.67 and 8.70 mg/100gm as hesperidine equivalent respectively, [1]. Total flavonoid content of lime (*Citrus aurantifolia*) 39.03 ± 0.20 mg/100g of fresh weight [22].The difference with our results might be due to different extraction method adapted and also due to different environmental conditions. The analysis of the content of total polyphenols in *Mentha aquatica L.* (450.25 mg/100gm) and *Mentha crispa L.* (296.89 mg/100gm) [18]. Meanwhile, the total phenolic compounds in the extracts for *Mentha spicata* (expressed as Gallic acid equivalents) varied between 5.91 ±0.12 and 39.47 ± 1.81 mg/g (On D.W) [7]. The total phenolic compounds (TFC) expressed as quercetin equivalents, varied from 198.0 ± 0.22 to 768.0 ± 0.02 mg quercetin equivalent/100gm. Also, [27] reported that the aqueous and methanolic extracts of *Mentha piperita* were analyzed for their phytoconstituents. The quantitative

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estimation of the content of total phenolics and flavonoids were 10.93, 23.43 and 5.850%, 17.38% for aqueous and methanolic extracts respectively.

Table (1): Chemical and physical constituents of fresh peppermint, lime and its extracts (on fresh weight).

Properties	Mint leaves	Lime Juice	Mint Extract	Lime Extract
Moisture %	79.42 ±0.464	90.55 ± 0.075	ND	ND
T.S %	20.58 ±0.464	9.45 ± 0.075	ND	ND
pH value	6.73 ±0.036	2.36 ± 0.01	7.01 ±0.031	2.42 ±0.050
Total acidity % (as citric acid)	0.205 ±0.015	5.83 ± 0.04	0.013 ±0.006	2.26 ±0.02
Ash %	1.71 ±0.078	0.29 ± 0.02	ND	ND
Total phenolic compounds (as Gallic acid).	360.04* ±0.285	193.02* ± 0.183	90.01** ±0.123	77.21** ±0.089
Total flavonoids compounds (as Quercetine)	421.96* ± 0.25	53.41* ±0.17	105.49** ±0.130	21.36** ±0.076
AOA % (antioxidant activity)	87.74 ±0.39	80.34 ± 0.74	88.05 ±0.185	41.30 ±0.155

*mg/100g, * * mg/100ml ND: not determined.
Values are means of three replicate determination ±standard deviations.

This slight difference from the results might be due to the different extraction method adapted and also due to different environmental conditions. The results in Table (1) illustrated that the antioxidant activity of peppermint leaves, peppermint extract, lime juice and lime extract was 87.74±0.39, 88.05±0.185, 80.34±0.74and 41.30±0.155 respectively. It is well known that plant phenols and flavonoids in general are highly effective free radical scavenging and antioxidants.

3.2 Sensory evaluation of peppermint, lime beverage and its treatments:

Blending could lead to the production of delightful and delicious beverages with improve organoleptic quality and high nutritive value. Blending increases taste and flavor of juice. The blending may also improve aroma, taste and nutrients of the beverages.

Table (2): Sensory evaluations of peppermint, lime beverages.

Treatments	Properties			
	Color	Taste	Odor	Overall acceptability
T1 (40:5:55)	7.7^{bc} ±1.059	7.0^{c} ±0.816	8.3^{b} ±0.675	7.6^{c} ±0.689
T2 (45:5:50)	8.4^{ab} ±0.699	8.4^{b} ±0.516	8.6^{b} ±0.518	8.7^{b} ±0.483
T3 (50:5:45)	9.3^{a} ±0.675	9.1^{a} ±0.738	9.4^{a} ±0.692	9.5^{a} ±0.527
T4 (55:5:40)	7.0^{cd} ±0.816	7.0^{c} ±0.667	6.9^{c} ±0.568	6.9^{d} ±0.738
T5 (60:5:35)	6.6^{de} ±1.265	6.3^{d} ±0.949	6.2^{d} ±0.919	6.3^{de} ±0.952
T6 (65:5:30)	6.0^{e} ±1.155	5.7^{de} ±0.823	5.7^{d} ±0.876	5.8^{ef} ±1.101
T7 Control 100 % mint extract	6.0^{be} ±1.333	5.3^{e} ±0.823	5.7^{d} ±0.675	5.6^{f} ±0.699

(v: v: v) of mint extract, lime juice and fizzy water respectively.
Values are means of three replicate determination ±standard deviations.
a-f means with a column with the different letters are significantly different.

When blending the peppermint extracts with the lime and soda water, the mixing is done with a view to elevate the properties of the original peppermint extract and avoid undesirable changes. Before blending, the extracts were examined in well-lit surroundings for clarity. Color has a major impact on the sensory properties of beverages, and has a significant

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effect on the ability to identify flavor or odor within a beverage, when color was appropriate for flavor or odor correct identification of the flavor was observed. The amount of extracts to be added, were determined by first trying-out on smaller quantities, until they produced desirable sensory effects. The results obtained from these trial additions, were followed for adding the extracts into the rest of the treatments.

Data in Table (2) revealed that the peppermint, lime beverage as well as those blended with fizzy water (T3) had the highest scores of color, taste, odor and overall acceptability followed by (T2). The products obtained a general overall acceptable score ranging from 5.6 to 9.5. Results in the same table ascertained that the treatments increased in addition of peppermint extract decreased in the sensory attributes for treatments control, T4, T5 and T6 blends. These results may be due to add the extract of peppermint can produce heavy flavors if used in large quantity. Such heavy flavors being undesirable in beverages, it was ensured that peppermint extract are taken in moderate quantity.

3.3 Physical and chemical constituents of peppermint, lime beverages:

The chemical compositions of the peppermint, lime beverage are given in Table (3). The T.S.S of peppermint, lime Beverage was 13.0 Brix This value according to [11]. Non-enzymatic browning (NEB) is one of the most important chemical reactions responsible for quality and color changes during the heating or prolonged storage leading to brown coloration, due to chemical reactions such as caramelization, ascorbic acid degradation and the Maillard reaction. It is the most common quality problem and causes loss of nutrients and the formation of intermediate undesirable compounds like furfural and 5-hydroxymethylfurfural (5-HMF). Data in the same table showed that color index (non enzymatic browning) of peppermint, lime beverage was ranged from 0.109 ±0.003 to 0.148 ±0.001. The pH was ranged from 3.30±0.024 to 3.68 ±0.042 and the total acidity% (as citric acid) was varied from 0.22±0.009 to 0.32±0.03%. Results in the same table (3) show the quantification of the extracted compounds for each sample (peppermint, lime Beverage). For the aqueous extracts, the results for flavonoid concentration showed that all samples presented statistical differences. Total flavonoids are determined spectrophotometrically with aluminum chloride ($AlCl_3$) and expressed as quercitien. The highest content of flavonoid was recorded in T6 (141.02 ±0.18 mg/100ml). In general, flavonoid contents of peppermint, lime beverage were significantly increased for all aforementioned treatments (Fig.1).

Table (3): Physicochemical characteristics of the peppermint, lime beverages.

Properties	Treatments						LSD at 0.05
	T1 40:5:55	T2 45:5:50	T3 50:5:45	T4 55:5:40	T5 60:5:35	T6 65:5:30	
T.S.S	13a ±0.02	13.1^{a} ±0.00	13.^{a} ±0.00	13.1^{a} ±0.01	13^{a} ±0.00	13^{a} ±0.03	0.11
Color index at(420nm)	0.109^{a} ±0.003	0.125^{b} ±0.002	0.126^{b} ±0.001	0.134^{c} ±0.001	0.142^{d} ±0.002	0.148^{e} ±0.001	0.003
pH	3.31^{d} ±0.006	3.30^{cd} ±0.024	3.42b^{c} ±0.07	3.47^{b} ±0.006	3.43b^{c} ±0.066	3.68^{a} ±0.042	0.069
Total acidity% (as citric acid)	0.29^{bc} ±0.01	0.32^{a} ±0.03	0.26^{c} ±0.03	0.26^{c} ±0.01	0.31^{ab} ±0.06	0.22^{d} ±0.009	0.033
Total flavonoids compounds mg/100ml (as Quercitien)	70.46^{f} ±0.45	80.67^{e} ±0.08	89.97^{d} ±0.19	103.51^{c} ±0.21	121.18^{b} ±0.21	141.02^{a} ±0.18	0.434
Total Phenolics compounds mg/100ml (as Gallic acid)	81.05^{a} ±0.152	78.39^{b} ±0.204	78.31^{b} ±0.148	71.80^{c} ±0.115	69.75^{d} ±0.125	69.13^{e} ±0.070	0.310
AOA % (antioxidant activity)	53.50^{f} ±0.11	53.84^{e} ±0.17	54.65^{d} ±0.05	55.54^{c} ±0.08	60.47^{b} ±0.21	70.07^{a} ±0.23	0.303

(v: v: v) of mint extract, lime juice and fizzy water respectively.
Values are means of three replicate determination ±standard deviations.
a-f means with a raw with the different letters are significantly different.

As for phenolic compounds (as Gallic acid) of the peppermint, lime beverage it could noticed that only significant differences were observed between (T1) and other treatments. Where, (T.P.C) was decreased significantly in (T2, T3, T4, T5 and T6). The highest content of phenolic compounds was recorded for T1 (81.05±0.152).While no significant

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differences between T2 and T3 on the total amount of phenolic compounds (Fig.1).[20] Reported that the effect of pH in the extraction of total phenols from meadowsweet herb (*Filipendula ulmaria L.*) was studied using water as solvent and it was verified that increasing the pH from 3.9 to 6.4 the total phenols content was higher.

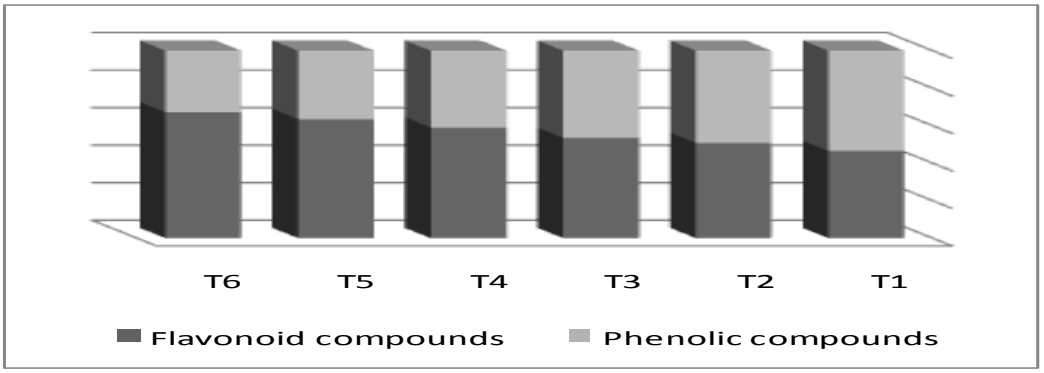

Fig (1): Total phenolic and flavonoid compounds in peppermint, lime beverages.

On the other hand, the efficiency of extraction from green tea was observed a decrease in the extraction efficiency when pH was increased, reaching a 30% less extraction at pH 6.0 and 15% at pH 7.6, [40]. The effect of pH variation could have a positive or a negative effect on phenolic compounds extraction, depending on the interaction of the polyphenols with other constituents of each plant.

DPPH assay DPPH scavenging method has been used to evaluate the antioxidant activity of compounds due to the simple, rapid, sensitive, and reproducible procedure. The antioxidant activity (AOA %) of peppermint, lime beverage is shown in Table (3). It was found that the increased mint extract ratio raised antioxidant activity. Therefore, the DPPH radical scavenging activity of these treatments depended on flavonoid content, not phenolic content similar to the result of [28] who reported that the DPPH radical scavenging activity of curry paste depended on flavonoid content not phenolic content, also [21] reported that phenolic acid had the lower antioxidant capacity than flavonoid. AOA% in peppermint, lime beverage of different treatments was in the range from 53.50±0.11to 70.07±0.23. Moreover, antioxidant activity, obtaining from water extraction was also equally high when compared with ethanol and methanol. [23] reported that water extraction of plant organs leaves a large amount of residual polyphenols that only an appropriate combination of solvents would extract. It appears that the vast majority of polyphenols are not water soluble. Therefore, to be assured of obtaining fractions rich in polyphenols manufacturers would have to use extraction solvents with a mixture of suitable solvents. The decrease in the antioxidant activity of the peppermint, lime beverages was due possibly to the transformation and oxidation of phenolic compounds.

3.4. Identification and quantification of phenolic compounds in peppermint, lime and their beverages:

Phenolic compounds are divided into two groups: flavonoids and phenolic acids. Phenolic acids are classified into two subgroups, hydroxybenzoic acids and hydroxycinnamic acids. Coumaric acid, caffeic acid, ferulic acid and cinnamic acid are examples of hydroxycinnamic acids; salicylic acid, p–hydroxybenzoic acid, gallic acid, protocatechuic acid and vanillic acid are examples to hydroxybenzoic acids [2].

Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain state of well being. Aqueous extract of peppermint and lime were analyzed for their phytoconstituents. Chemical investigations by HPLC indicated the presence of many biologically active constituents in the extracts of both mint and lime. The major phenolic constituents are caffeic acid derivatives and flavonoids by [33], [35]. The major phenolic components of the *Lamiaceae* family of herbs: rosmarinic acid, caffeic acid, protocatechuic acid, catechin, catechol, coumaric acid and quercetin had strong α-glucosidase [39].

A total of 13 phenolic compounds was identified and quantified in peppermint, lime and its beverages including hydroxybenzoic acids and hydroxycinnamic acids. As can be seen in Table (4) the phenolic content of the mint extract, lime extract, and the peppermint, lime beverages determined by the Folin – Ciocalteu method was higher than the concentration obtained by HPLC method. This can be explained by the lack of selectivity of Folin- Ciocalteu reagent,

which reacts not only with phenols but also with other reducing compounds such as carotenoids, amino acids, sugars and vitamin C [37] **,** [13].

Hydroxybenzoic acids, seven different hydroxybenzoic acids (Ellagic, aminobenzoic, vanillic, p-hydroxybenzoic, protocatechuic, salicylic and gallic acid) were detected and quantified in the all samples (Table 4). Peppermint extract presented the highest hydroxybenzoic acid content. ellagic acid accounted for the largest proportion (8.162 mg/100ml) of the total hydroxybenzoic acid contents. The highest level of aminobenzoic acid was detected in lime extract (5.015 mg/100ml). Also, the vanillic acid content in peppermint extract had the highest (5.169mg/100ml), while lime extract had the lowest (1.509 mg/100ml) contents. P-Hydroxybenzoic acid and protocatechuic acid content were the ranged from 3.054 to 4.008 and 2.659 to 2.717 mg/100ml for the mint and lime extracts respectively, salicylic and gallic acid content were ranged from 0.460 to 0.480 and 0.258 to 0.358 mg/100ml for the mint and lime extracts respectively.

As for Hydroxycinnamic acids, four hydroxycinnamic acids were identified in the analysis were caffeic, chlorogenic, cinnamic acid coumarin. Data in (Table 4) observed that caffic acid was the most dominant hydroxycinnamic acid in mint extract and lime extract (18.131–8.779mg/100ml) respectively, [35]. Chlorogenic acid (2.944 - 6.902mg/100ml) was the second most abundant hydroxycinnamic acid, followed by caffeic for mint and lime extracts respectively. Meanwhile, the mint and lime extracts contained catechol and pyrogallol acids in quantities of up to 2.317, 2.606 and 2.513, 2.641 mg/100ml, respectively.

Table: (4). Identification and quantification of phenolic acids in peppermint, lime and their Beverages (mg/100ml).

Phenolic Compound mg/100ml	Treatments							
	Peppermint Extract	Lime Extract	T1 40:5:55	T2 45:5:50	T3 50:5:45	T4 55:5:40	T5 60:5:35	T6 65:5:30
Ellagic	8.162	2.071	3.314	9.426	6.224	7.449	3.430	1.091
Aminobenzoic	6.443	5.015	2.930	2.825	1.859	2.343	1.484	1.150
vanillic	5.169	1.509	4.678	4.129	4.075	3.316	3.483	1.401
P hydroxy benzoic	4.008	3.054	3.611	1.854	5.508	3.014	3.399	2.264
Protocatechuic	2.717	2.659	2.069	2.515	1.823	1.926	2.415	1.163
Salicylic	0.480	0.460	0.232	0.270	0.428	0.208	0.110	0.120
Gallic	0.358	0.258	0.523	0.667	0.550	0.639	0.217	0.327
Caffeic	18.131	8.779	5.140	19.777	12.426	9.391	8.713	6.267
Chlorogenic	2.944	6.902	0.848	0.501	0.753	0.578	1.873	0.580
Cinnamic	1.115	0.334	0.620	1.217	0.429	0.496	0.000	0.259
Coumarin	0.657	0.725	0.461	0.295	0.953	0.394	0.232	0.208
catechol	2.317	2.513	2.213	3.736	3.202	2.135	1.869	1.747
pyrogallol	2.606	2.641	0.324	2.605	2.807	2.086	1.878	1.712
Total	55.107	36.92	26.963	49.817	41.037	33.975	29.103	18.289

(v: v: v) of mint extract, lime juice and fizzy water respectively.

Data in the same table (4) showed that the highest content of ellagic acid was 9.426 mg/100ml for (T2) followed by T4 (7.449 mg/100ml) and T3 (6.224 mg/100ml) and the lowest concentration found in T6 (1.091 mg/100ml). Treatment (T2) had high concentration of caffeic acid (19.777 mg/100ml) while T3 had moderate content of caffic acid (12.426 mg/100ml). T2 and T3 content the highest amounts of catechol acid 3.736 and 3.202 mg/100ml respectively than other ones. While, pyrogallol acid concentration was 2.807 mg/100ml for T3 followed by (T2, T4, T5, T6 and T1) respectively. Generally, it was observed that the concentration obtained by HPLC for phenolic compounds was identified and

quantified.T2 contained high levels of phenolic (49.817 mg/100ml) followed by T3 (41.037 mg/100ml) while T6 contained low levels of phenolic (18.289 mg/100ml).

Generally, it was observed that quantitative and qualitative differences for the phenolic compounds generated by the blend process may be attributed, at least in part, to the variation in phenolic compounds for each other. Some of the new compounds formed during processing of beverages are often overlooked in studies addressing food composition, although they may show particular properties different from their precursors [9]. These changes were possibly due to the transformation of phenolic compounds into condensed forms that possessed slightly different chemical properties. These differences may be due to variations of the ratio of blends.

3.5 Identification and quantification of flavonoid compounds in peppermint, lime and their beverages (mg/100ml):

Flavonoids have six subgroups due to difference of the connection between two aromatic rings called as anthocyanidins, flavanols, flavones, flavanons, flavanols and isoflavones, [29]. Flavanols are colourless and water soluble compounds and take place as "mid product" in flavonoid biosynthesis. They can easily condense with oxygen both chemically and enzymatically and form proanthocyanidins. Flavanols can scavenge free radicals in vitro and in vivo. Physiologically foods and beverages rich in flavanols can influence platelet aggregation, vascular inflammation and endothelial nitric oxide metabolism and may confer protective effects against neurodegeneration [19].

The extracts of peppermint leaves, lime juice and other beverage were further analyzed by high performance liquid chromatography (HPLC) to identify and quantify major bioactive flavonoid compounds profile. Data revealed that naringin, rutin, quercitrin and hesperetin were the dominant content in peppermint extract (23.005, 14.269, 15.369 and10.937mg/100ml) respectively, followed by hesperidin, rosmarinic, naringenin and quercetin (5.070, 4.375, 4.399 and 0.643 mg/100ml) respectively. Different bioactive flavonoid compounds, including rutin and naringenin were obtained from spearmint (*Mentha spicata L.*) rutin and naringenin content 16.1 and 4.50 mg/100g, respectively [5].

Table (5): Identification and quantification of flavonoid compounds in peppermint, lime and their beverages (mg/100ml).

Flavonoid Compound mg/100ml	Treatments							
	Peppermint Extract	Lime Extract	T1 40:5:55	T2 45:5:50	T3 50:5:45	T4 55:5:40	T5 60:5:35	T6 65:5:30
Naringin	23.005	0.846	10.817	7.820	9.644	9.489	6.426	7.407
Rutin	14.269	1.182	3.647	24.919	22.626	7.311	11.326	4.281
Hesperidin	5.070	0.393	4.342	7.948	3.279	9.250	2.946	2.326
Rosmarinic	4.375	0.513	0.959	2.894	1.816	1.864	0.851	0.561
Quercitrin	15.369	0.447	5.337	5.390	8.583	6.931	4.757	3.054
Quercetin	0.643	0.048	0.566	1.106	1.181	0.567	0.368	0.207
Naringenin	4.399	0.026	0.797	2.632	2.160	1.865	1.627	1.294
Hesperetin	10.937	0.289	0.000	15.542	10.046	0.000	7.119	4.931
Total	78.067	3.744	26.465	68.251	59.335	37.277	35.42	24.061

(v: v: v) of mint extract, lime juice and fizzy water respectively.

The flavonoid compounds were identified and quantified (by HPLC) in lime juice and found the highest decreased concentration than peppermint extract. Flavonoids compounds such as hesperidin, narirutin and rosmarinic were identified in an aqueous extract (Ex) obtained from peppermint leaves [33], [38] found that most phenolic compounds contained in citrus juices were flavonoid glycosides including hesperidin, narirutin, naringin, neohesperidin. Also, [8] reported that several flavonoids including hesperidin, quercetin derivatives in lime (*Citrus aurantifolia*).

This HPLC analysis revealed the presence of some major flavonoid compounds such as rutin, naringin, hesperetin. quercitrin and hesperidin as major compounds in peppermint, lime beverages followed by naringenin, rosmarinic and

quercetin. The investigation revealed that the highest level of flavonoid rutin, hesperetin and naringenin contents were 24.919, 15.542 and 2.632 in T2, while they recorded 22.626, 10.046 and 2.160 ml/100ml for T3 respectively. On the other hand T1 had maximum content from naringin content (10.817 ml/100ml). Meanwhile, T4 could be considered high level of hesperidin (9.250mg/100ml) followed by T2 (7.948 mg/100ml). As well as T3 had a maximum concentration from quercitrin (8.583 mg/100ml). Rosmarinic and quercetin were the least abundant flavonoids in the samples, ranging from 0.561 to 2.894 mg/100ml and from 0.207 to 1.181 mg/100ml in all treatments. Flavonoid and phenolic quantitative results were compared in order to choose which combination of mint extract, lime juice and soda water the best one to produce a novel mint lime beverage extract.

Generally, from aforementioned results identified and quantified (by HPLC) (Tables 4 and 5) revealed that the treatments differed in their content of some bioactive and therapeutics phenolic compounds and found the following decreasing concentration, similar to the one observed in the present research: T2 > T3> T4 >T5> T1> T6.

4. CONCLUSION

In this work that extracts from those peppermint extract with lime juice have high polyphenolic content and antioxidant activity and therefore could be useful as nutraceuticals or antioxidant supplements against oxidative stress and ageing. The combination of peppermint extract with lime juice and carbonated water could be used to produce natural carbonated beverage.

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