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PHYTOCHEMICAL CONSTITUENTS AND THE DISTRIBUTION OF OXYCAROTENOIDS IN DEHYDRATEDGREEN LEAFY VEGETEABLES OF DIFFERENT SOURCES

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ABSTRACT

Plants possess many phytochemicals such as carotenoids, ascorbic acid,α-tocopherol, flavonoids, chlorophylls and polyphenols, etc. Green vegetables are packed with antioxidants, nutrients and fibers. These are essential for maintaining various biochemical processes including reducing levels of LDL-cholesterol, improving vision, helping to manage weight and boosting immunity. This study was undertaken to determine the total content of polyphenols, flavanoids, chlorophylls and xanthophylls in 15 different leafy vegetables from different regions by spectrophotometric method.The distribution of major oxycarotenoids such as lutein, zeaxanthin and β-cryptoxanthin was also studied using high performance liquid chromatography. The analytical data showed that the total polyphenol content ranged from 0.79-7.3 g/100g;total flavonoid content ranged from 1.1-23.7 g/100g;total chlorophyll content ranged from 0.47-4.7g/100g and the total xanthophyll content ranged from 0.17-1.65g/100g.In the oxycarotenoid distribution the results showed that lutein content of leafy vegetables ranged from 0.03-0.64 g/100g.The analysis also showed the presence of zeaxanthin and β-cryptoxanthin in some leaves like coriander curry leaf, fenugreek(methi) and mint leaves.Furthermore, we can conclude that the selected 15 different leafy vegetables are nutritionally and medicinally important and those of Indian sources are rich in xanthophylls whereas of Egyptian sources are rich in polyphenols and flavanoids

KEYWORDS: *Green leafy vegetables, polyphenols, flavonoids, chlorophylls, xanthophylls*

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INTRODUCTION

Green leafy vegetables are the cheapest of all the vegetables within the reach of poor man, being richest in their nutritional value[1].Green leafy vegetables are used since ancient periods as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. Plant foods are sources of energy, micronutrients and nutrients essential to health, in addition to phytochemicals with further health benefits including glycemic control, immune-stimulation or antioxidant activitvity[2,3]. Green leafy vegetables provide fiber,vitamins and minerals. Moreover, these have been identified as good sources of natural antioxidants like tocopherols, vitamin C and polyphenols which help in for maintaining good health and also protect against coronary heart diseases and cancer. Phytonutrients present in green leafy vegetables produce many common health benefits like protection from eye problems, oxidative stress, iron deficiency, etc[4,5]. Carotenoids are important constituent of green leafy vegetables[6]. Besides chlorophylls and anthocyanins, carotenoids belong to the most important and most frequent pigments found in nature, the main sources being yellow, orange, and red fruits and vegetables as well as green leafy vegetables. Carotenoids, fat soluble, colored pigments serve a variety of functions in cellular biology. Carotenoids (β-carotene, lycopene and lutein) present in fruits and vegetables have antioxidant functions such as scavenging of singlet oxygen and other electronically excited molecules and also reduce the progression of many degenerative diseases[7,8].Antioxidants that neutralize free radicals are also secondary constituents or metabolites found naturally in the body and in plants such as fruits and vegetables. Plants contain an array of antioxidant compounds such as carotenoids, flavonoids, related polyphenols, cinnamicacid, benzoicacid, folic acid, ascorbicacid, tocopherols and tocotrienols which prevent oxidation of the susceptible substrate[9]. Common antioxidants include vitaminA, vitaminC, vitaminE and certain compounds called carotenoids (like lutein and β-carotene)[10]. Free radicals such as peroxide, hydroperoxide or lipidperoxyl are scavenged by polyphenols and flavonoids thereby inhibit the oxidative mechanisms[11]. Polyphenols scavenge free radicals (R*) possessing an unpaired electron either by donation of hydrogen or electrons, resulting in comparatively stable phenoxyl (PhO*) radicals (neutral (PhO*)or cationic (PhO+*) molecules, respectively), which are stabilized by delocalization of unpaired electrons around the aromatic ring[12,13a,13b]. Carotenoids are natural pigments and the pattern of conjugated double bonds in the polyene backbone of carotenoids determines their light absorbing properties and influences the antioxidant activity of carotenoids[14,15]. The antioxidant action of carotenoids is their ability to quench singlet oxygen via physical or chemical quenching. According to the chemical composition,carotenoids are categorized as either carotenes or xanthophylls(oxycarotenoids); the carotene group,such as β-carotene, α-carotene and lycopene,composed only of carbon and hydrogen atoms,xanthophylls such as zeaxanthin, lutein, α and β-cryptoxanthin, carry atleast one oxygen atom[16].Oxygenated carotenoids, including lutein, which have little or no pro-vitamin A activity, have been recognized to have antioxidant activities[17]. Singlet oxygen generated in biological systems is capable of damaging proteins, lipids and DNA. Lutein and zeaxanthin absorb visible light and play a role in singlet–singlet energy transfer and the quenching of singlet oxygen[18,19]. This ability may contribute to the protection of light-exposed tissue, skin and eyes, from light-induced damage[20].The present study was conducted with the objective to quantify the phytochemical constituents and the distribution of oxycarotenoids in the dried green leafy vegetables which are commonly consumed in India, Egypt and Thailand.

MATERIALS AND METHODS

MATERIALS

Sample collection

Dehydrated green leafy vegetables of Curry leaves,Methileaves,Corianderleaves,Broccoli leaves are collected from South East Asia(Farm to Home,Banglore); Bay,Oregano, Marjoram,Basil, Pandan ,Sage and Thyme from Egypt regions(Pharaohs Herbs,Export herbs and spices,Egypt Invoice No.85/2015 & 4/2016);Lemongrass,Kaffirlime,and Parsley leaves are from Thailand (Chaicahda Co Ltd,,Bangkok;Invoice No CCDHBPD17031-006062 & 006085) region.

Solvents

Methanol, Ethyl alcohol, Acetone, Hexane, Ethyl acetate, Toluene were purchased from Merck.

Reagents

Folin and Ciocalteu,Sodium Nitrite,Aluminium chloride, Potassium hydroxide, Sodium carbonate and Sodium sulphate were also purchased form Merck.

Standards

Gallic acid and Quercetin were purchased from Sigma. Carotenoid standards such as Lutein, Zeaxanthin, β-carotene and β-cryptoxanthin were received from Omni Active Health Technologies, Mumbai.

All solvents were checked fortheir purity by GC Analysis. High purity water was prepared with a Millipore water purification system.

METHODS

Sample preparation

The powdered leafy material of 0.5 g was refluxed twice with 5 ml methanol and 50 ml water for 1 hr at 80^0C.Cooled and filtered quantitatively to a 100ml volumetric flask and diluted with water. 5ml of the aliquot was pipetted into 25ml volumetric flask and diluted with water.

Total Polyphenol Content

The total polyphenols in dried leaves was determined using spectrophotometric method[21]. The color development was carried out by mixing 1ml of the sample solution, 1 ml of Folin-Ciocalteu reagent,15ml water and 3ml of saturated $NaHCO_3$

solution. Blank was concomitantly prepared,containing 1 ml 70% methanol,1 ml Folin-Ciocalteu reagent,15ml water and 3 ml of saturated $NaHCO_3$ solution. The samples were heated in a thermostat at 45°C for 45 min. The absorbance was measured at 750 nm. The same procedure was repeated for the standard solution of gallic acid . Based on the measured absorbance, the polyphenols are calculated and expressed in terms of gallic acid equivalent .

Total Flavonoid Content

The total flavonoids in dried leaves was determined using spectrophotometric method[22]. The reaction mixture was prepared by mixing 1ml of the sample solution, 0.3 ml of 5% sodium nitrite, 0.3 ml 10%Aluminium chloride,2ml 1M sodium hydroxide and 15 ml water.Blank was concomitantly prepared,containing 1 ml 70% methanol, 0.3 ml of 5% sodium nitrite,0.3 ml 10%Aluminium chloride,2 ml 1M sodium hydroxide and 15 ml water. The absorbance was determined using spectrophotometer at 510 nm. The same procedure was repeated for the standard solution of quercetin . Based on the measured absorbance, the flavonoids are calculated and was expressed in terms of quercetin equivalent .

Total Chlorophyll Content

The content of total chlorophyll in dried leaves was determined using spectrophotometric method[23]. The powdered leafy material 3g was sonicated 4-5 times with 50 ml methanol each time for 20 minutes. Filtered into a 250 ml standard flask and made up to the mark using methanol. 5ml of the aliquot was further diluted to 50ml with acetone. The absorbance was noted at 410nm, 430nm, 642.5nm, 660nm and 663nm .

Total Xanthophyll Content

The content of total xanthophylls and their distributions in dried leaves was determined using spectrophotometric method and HPLC methods[23].

Extraction Of Oxycarotenoids from dried leaves

A representative sample of the grinded leafy sample 3 g was refluxed with 10 ml Hexane,6 ml Ethanol, 6 ml Acetone,7ml Toluene and 2 ml 40% methanolic KOH in a waterbath kept at 56°C +/- 0.5°C for 20 minutes.The sample was cooled and 30 ml hexane was added and diluted to the mark with 10% sodium sulphate solution.Allowed to stand in the dark for 1 hour for the phase separation to take place . Pipetted 1 ml of aliquot from the upper phase in to 50 ml volumetric flask and diluted to the mark with the solvent hexane: acetone: methanol(8:1:1).The absorbance of the solution was measured at 474 nm for total xanthophylls and 5 ml of the aliquot was taken from the upperphase, filtered the solution using 0.22 micron Millipore filter, injected 20 micro liter of the solution into HPLC.Individual standards of carotenoids were also prepared by dissolving the respective standards in ethylacetate and made 50-100ppm solution.

HPLC Instrumentation

The analysis of the dried leaves and carotenoid standards were carried out by normal phase HPLC analysis.The Agilent Technology 1260 HPLC system equipped with DAD, gradient pump, a degasser, column thermostat and an autosampler. The separation was carried out in a Kromasil silica analytical column 250 x 4.6 mm id; particle size 5 µm with a Silica guard column used for normal phase analysis.The mobile phase consisted of hexane and ethyl acetate (75/25). All solvents were filtered through a syringe filter prior to the analysis. The flow rate was 2 ml /min for total run time 45 min., the detector was set 445 nm and Chemstation software was used for data processing and the injection volume was 20 uL.

RESULTS AND DISCUSSION

Table 1 depicts data on the analysis of phytochemical constituents such as polyphenols,flavonoids,chlorophylls and xanthophyll content measured by Spectrophotometry.Table 2 shows the distribution of oxy carotenoids like beta cryptoxanthin, lutein and zeaxanthin by normal phase chromatography.Table 3 represents the list of screened leafy vegetables with details of its family,scientific name and its medicinal properties.Figures 1,2 and 3 show the absorption spectrum of carotenoids and chlorophylls ; Overlaid chromatograms of individual standrds such as lutein,zeaxanthin, β-cryptoxanthin and typical HPLC profile of dried methi leaf respectively

Table 1
Phytochemical constituents such as Polyphenols, Flavanoids, Chlorophyll and Xanthophyll content in the dehydrated green leafy vegetables

No:	sample name	Total Polyphenol Content (g/100g)	Total Flavanoid Content (g/100g)	Total Cholorophyll Content (g/100g)	Total Xanthophyll Content (g/100g)
1	Basil	7.28	23.72	0.4605	0.4447
2	bay leaves	2.88	6.83	0.6830	0.1767
3	Broccoli	1.06	2.20	3.8822	0.6884
4	coriander	0.79	2.64	4.7647	1.6545
5	curry leaf	4.25	6.42	0.9495	1.3267
6	kaffir lime	1.50	2.64	1.4170	0.5287
7	lemon grass	0.91	1.66	0.8894	0.1265
8	Marjoram	5.50	19.18	1.0836	0.1617
9	Methi	1.84	4.44	3.9406	1.4519
10	Mint	7.01	22.50	4.7737	0.2942
11	Oregano	6.40	11.02	0.4797	0.1488
12	pandan leaves	1.04	1.05	1.9484	0.5693
13	parsely leaves	1.04	0.92	1.6262	0.2176
14	Sage	6.60	11.63	0.9775	0.4360
15	Spinach	0.84	2.08	4.4609	1.2021
16	Thyme	4.99	21.95	1.3167	0.3953

Table 2
Distribution of Oxycarotenoids such as Lutein,Zeaxanthin and β-cryptoxanthin content in the dehydrated green leafy vegetables by HPLC

No:	sample name	Lutein Content (g/100g)	Zeaxanthin Content (g/100g)	β-Cryptoxanthin Content (g/100g)
1	Basil leaves	0.123305	0.01368	0
1	Bay leaves	0.081731	0	0
2	Broccoli leaves	0.280396	0	0
3	Coriander leaves	0.641668	0.044655	0.001145
4	Curry leaves	0.611147	0	0.001196
5	kaffir lime leaves	0.203332	0	0
6	lemon grass	0.037542	0	0
7	Marjoram leaves	0.080173	0.013459	0
8	Methi leaves	0.546312	0	0.002617
9	Mint leaves	0.081888	0.023284	0
10	Oregano leaves	0.065739	0.016384	0
11	pandan leaves	0.194324	0	0
12	parsely leaves	0.052948	0.019797	0
13	Sage leaves	0.201082	0	0
14	Spinach leaves	0.483692	0	0
15	Thyme leaves	0.169247	0.032754	0

Table3
List of dehydrated green leafy vegetables with their Local name, ScientificName,and Health benefits

Sl No.	Local name	Scientific Name	Major Health benefits*
1	**Basil**	*Ocimum basilicum*	Oral care, relief from respiratory disorders, treatment of fever, asthma, lung disorders, heart diseases and stress ,controlling blood glucose levels and cholesterol levels
2	**Bay leaf**	*Laurusnobilis*	Detoxify the body, slow the aging process, speed wound healing, protect the body from bacterial infections, manage diabetes, improve heart function, reduce inflammation, alleviate respiratory problems, optimize digestion, and prevent certain types of cancer.
3	**Broccoli**	*Brassica oleracea var. italic*	Anti-cancer, especially in the prevention and treatment of gastriccancer, breast cancer
4	**Coriander**	*Coriander sativum*	Skin inflammation, high cholesterol levels, diarrhea, mouth ulcers, anemia, indigestion, menstrual disorders, smallpox,conjunctivitis,skin disorders, and blood sugar disorders also benefiting eye care.
5	**Curry leaves**	*Murraya koenigii*	Anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, anti-carcinogenic and hepato-protective
6	**Kaffir lime**	*Citrus hystrix*	Oral health, detoxify the blood, boost skin health, improve digestion, ward off insects, lower inflammation, aid the immune system, reduce stress, and improve the health of the hair relief from anxiety and stress
7	**Lemon grass**	*Cymbopogon*	Relief from stomach disorders, insomnia, respiratory disorders, fever, aches, infections, rheumatism and edema, helps in maintaining optimum cholesterol levels, cellular health, nervous system, healthy skin and immune system. Stopsdiarrhea
8	**Marjoram**	*Origanum majorana*	Relieving digestive problems, efficient sedative, stimulates lactation and perspiration, helps in flushing toxins from the body and anti-inflammatory
9	**Methi leaves**	*Trigonella foenum-graecum*	Aromatic,cooling,laxative, skin andmucousmembranes, relieve irritationof theskin, alleviate swelling andpain and diabetes

10	**Mint leaves**	*Mentha piperita*	Proper digestion and weight loss, relief from nausea, depression, fatigue and headache, treatment of asthma, memory loss, and skin care problems
11	**Oregano leaves**	*Origanum vulgare*	Respiratory tract disorders, gastrointestinal (GI) disorders, menstrual cramps, urinary tract disorders and rejuvenation
12	**Pandan leaves**	*Pandanus amaryllifolius*	Relieves gastrointestinal cramps and spasms, gives energy to new mothers, provides calming or soothing effects, lowers blood pressure, reduces fever and detoxifies the body
13	**Parsely leaf**	*Petroselinum crispum*	Controlling cancer, diabetes, and rheumatoid arthritis, along with helping prevent osteoporosis., relief from gastrointestinal issues and strengthen the immune system
14	**Sage**	*Salvia officinalis*	To improve brain function, lower inflammation, prevent chronic diseases, boost the strength of the immune system, regulate proper digestion, alleviate skin conditions, increase the health and strength of bones, prevent the onset of diabetes and lowers cholesterol
15	**Spinach**	*Spinacia oleracea*	Anti-oxidant,antiproliferative, anti-infammatory,antihistaminic, CNSdepressant, protectionagainstgamma radiation and hepatoprotective effect
16	Thyme	*Thymus vulgaris*	Reduce respiratory issues, boost the strength of the immune system, protect against chronic diseases, stimulate blood flow, prevent fungal infections, improve heart health, and relieve stress fights sore throats

(*source'Useful Plants Of India";;"Encyclopedia of Medicinal Plants" and www,organicfacts,com)

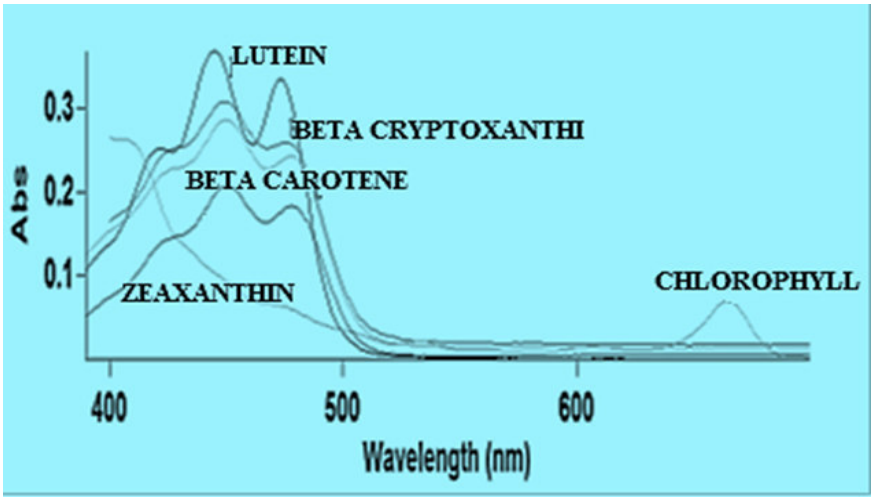


Figure 1
Characteristic UV-Vis spectrum of Carotenoids (Lutein, zeaxanthin, beta carotene, beta cryptoxanthin) and chlorophyll

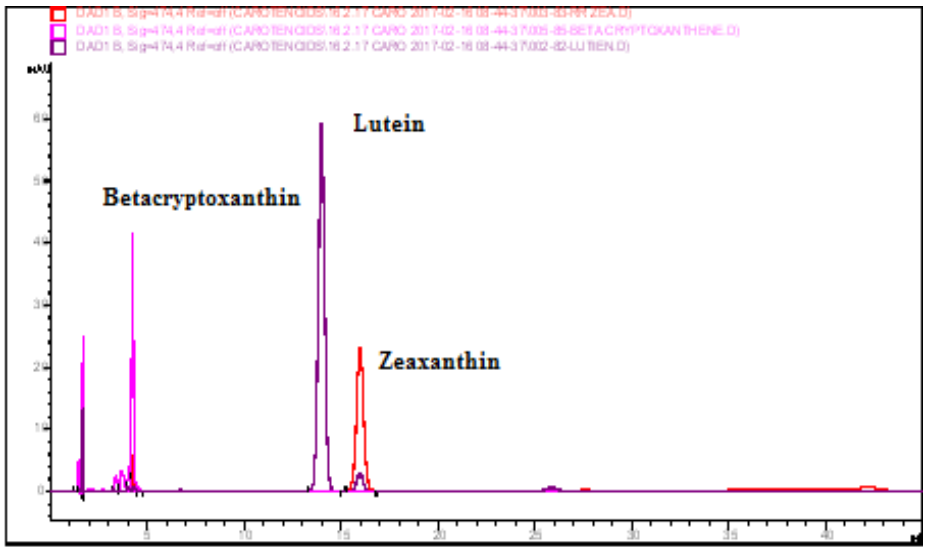


Figure 2
HPLC Chromatogram of Mixed standards

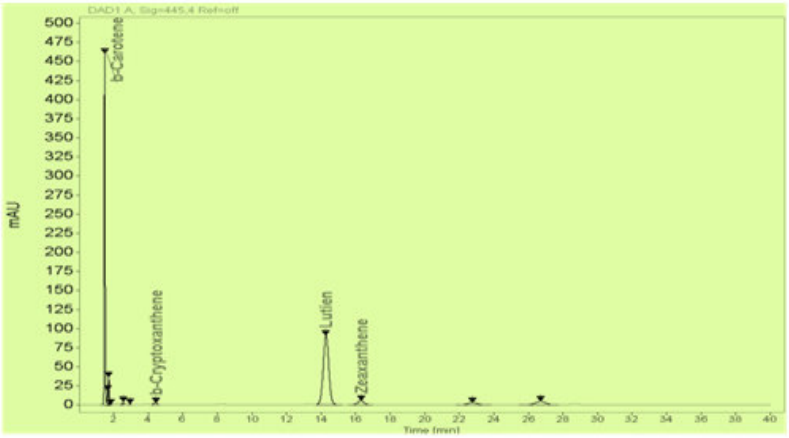
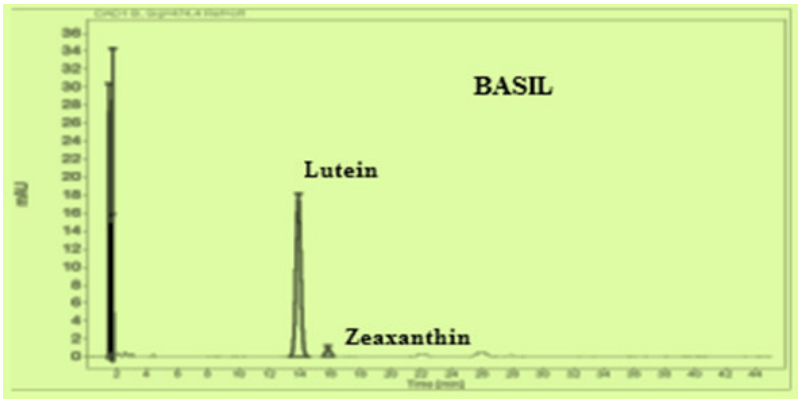
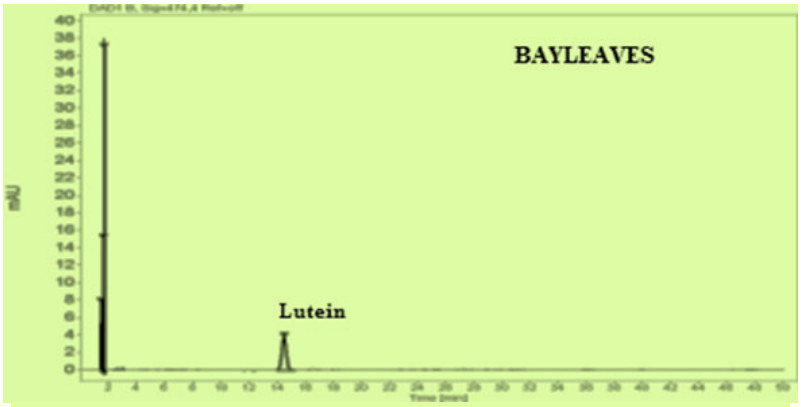
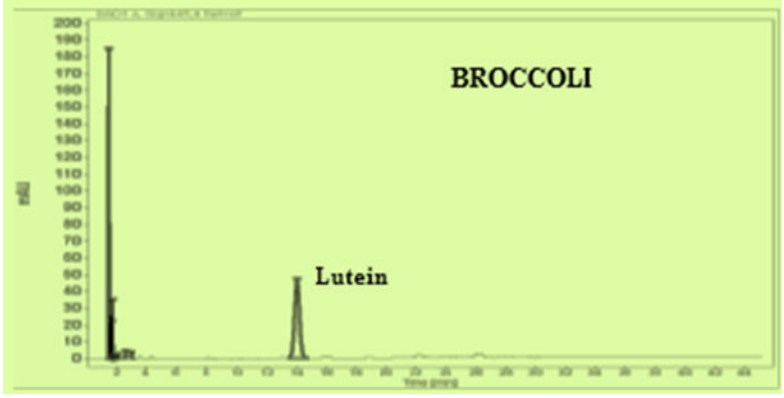


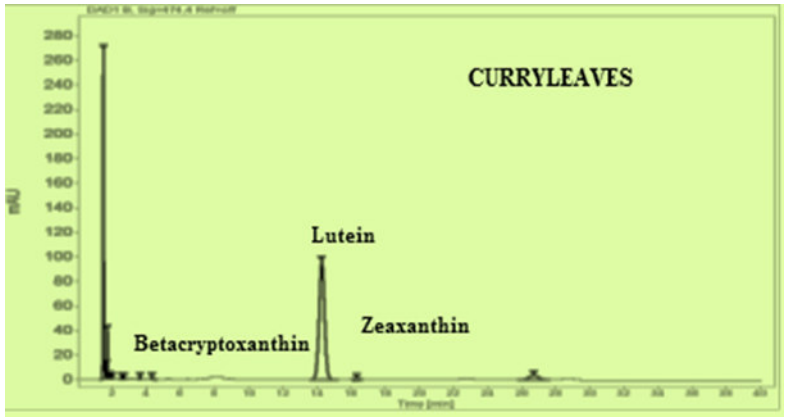
Figure 3
Typical HPLC profile of methileaves

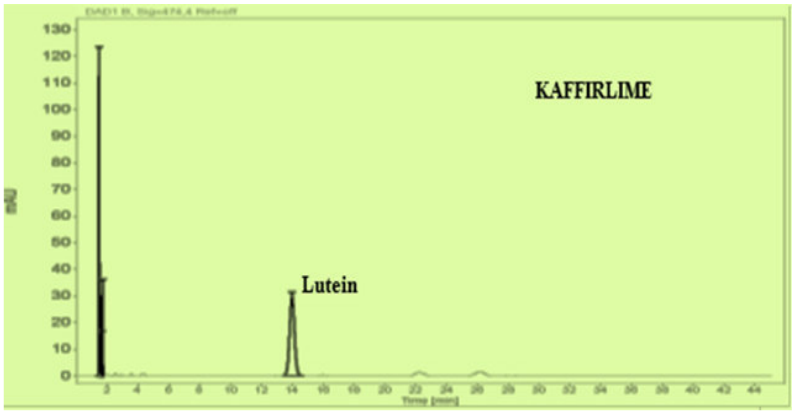
APPENDIX:HPLC chromatogram of the selected leaves

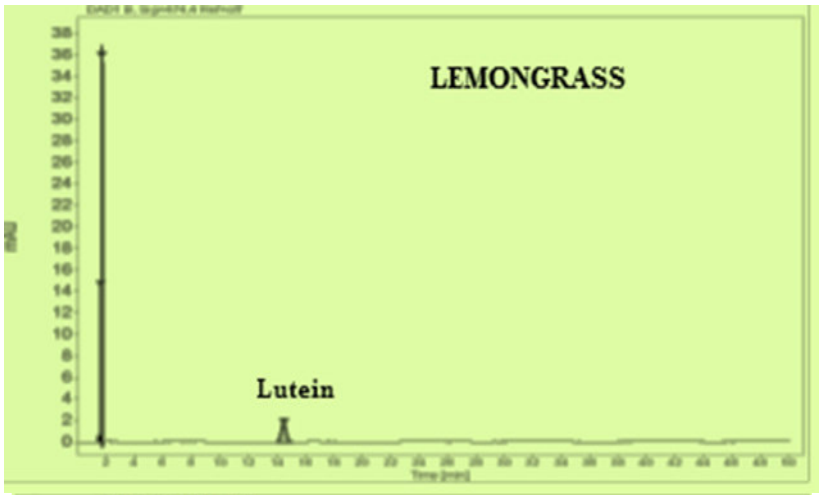







CURRYLEAVES
Lutein
Zeaxanthin
Betacryptoxanthin
mAU


KAFFIRLIME
Lutein
mAU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Lutein
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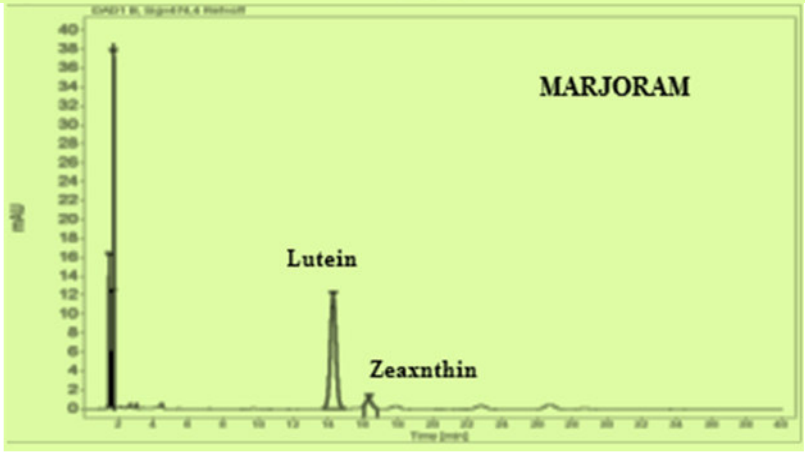
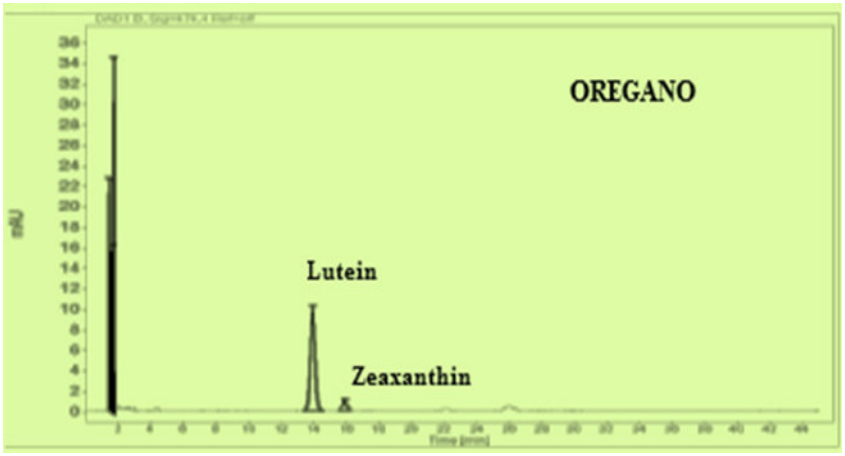
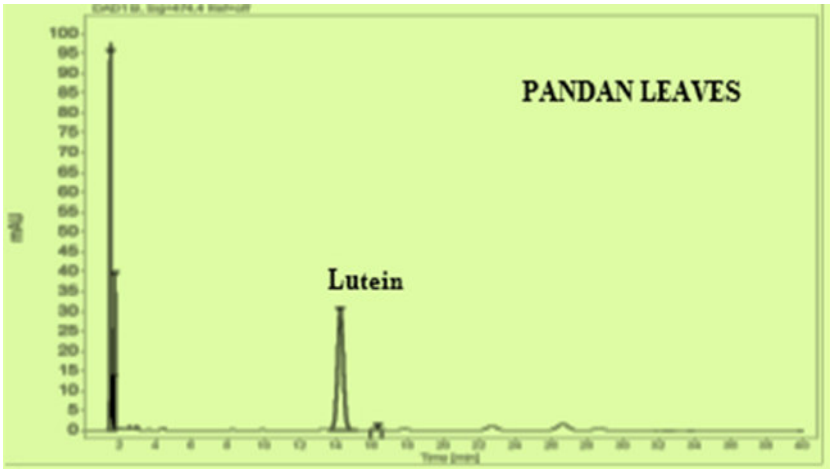
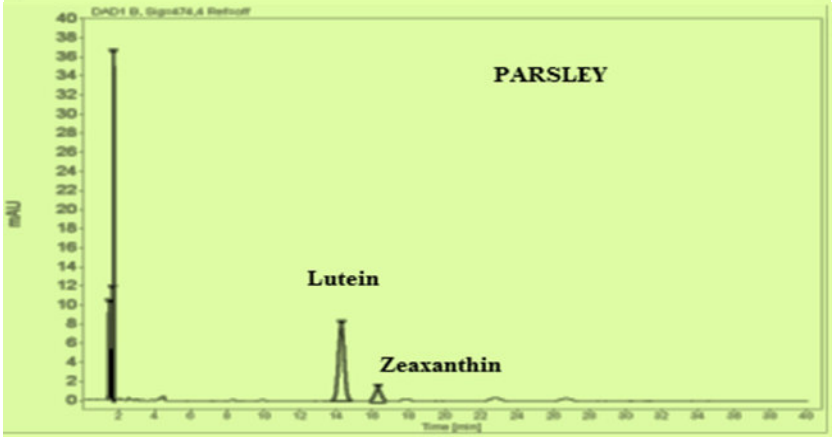
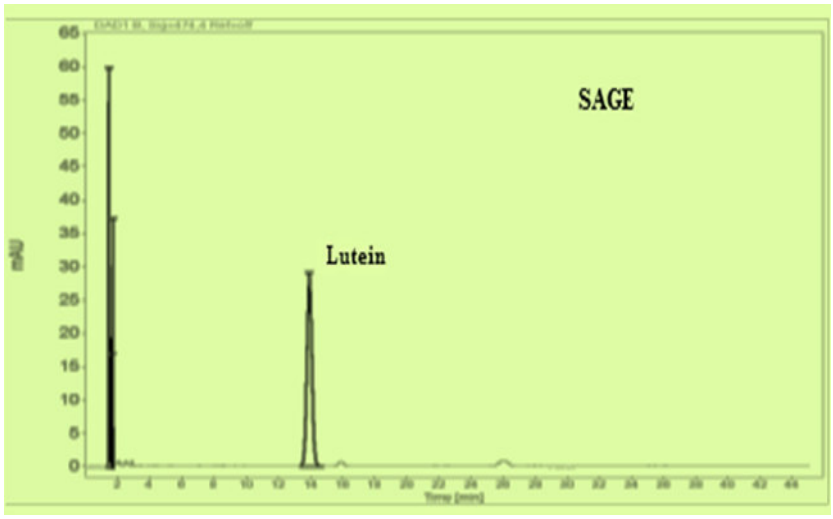

MARJORAM
Lutein
Zeaxnthin
mAU

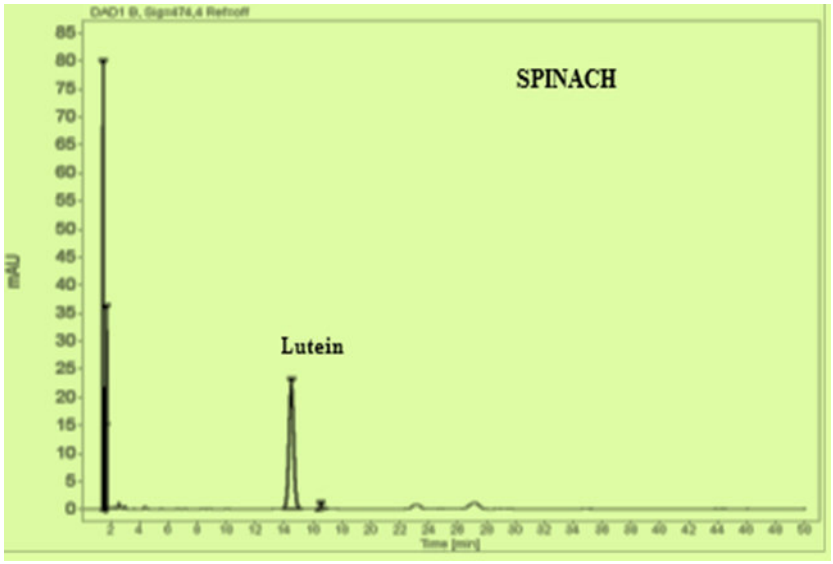

MINT LEAVES
Lutein
Zeaxanthin
mAU

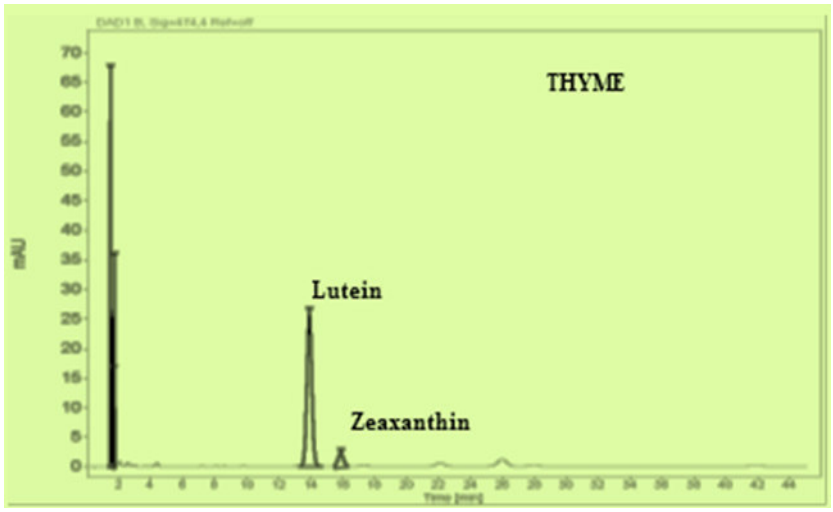

OREGANO
Lutein
Zeaxanthin
mAU


PANDAN LEAVES
Lutein
mAU


PARSLEY
Lutein
Zeaxanthin
mAU







The results of phytochemical constituents are presented in Table 2.From table 2 it is clear that the total polyphenols are in the range of 5-10% in basil, marjoram, mint, oregano and sage whereas in bayleaves, broccoli, curryleaves, kaffirlime, methi, pandan and parsley leaves are in between 1-5% and others such as coriander,lemongrass and spinach are showing less than 1%.The total flavonoids in dried leaves such as basil, marjoram, mint, oregano, sage and thyme are in the range of 10-24%; bayleaves and curryleaves are showing 5-10% and broccoli, coriander, methi, kaffirlime, lemongrass, pandan, parsley and spinach are below 5%.Total chlorophyll contents are showing 3-5% in broccoli, coriander, methi, mint and spinach;1-5% in kaffirlime, marjoram, parsley, pandan leaves and thyme;below 1% in basil, bayleaves, curryleaves, lemongrass, oregano and sage. Total xathophylll contents are showing between 1-2% in coriander, curryleaves, methi and spinach;0.5-1% in broccoli, kaffirlime and pandan leaves and others such as basil, bayleaves, lemongrass, marjoram, mint, oregano, parsley and thyme are below 0.5%.The data in

table 3 shows the content of catotenoids, in which lutein content in coriander, curryleaves and methi leaves are in the range of 0.5-1% while in broccoli, kaffirlime, spinach, sage are in the range of 0.2-0.5% and others like basil, bayleaves, lemongrass, marjoram, mint, oregano, parsley and thyme are 0.05-0.2%.Zeaxanthin content in basil, coriander, marjoram, mint, oregano, parsley and thyme are in the range of 0.01-0.08% and in others no zeaxanthin was observed. In most of the dried leaves betacyrptoxanthin was not showing any peak but in coriander, curry leaves and methi shows 0.00115,0.00015,0.0026% respectively.

CONCLUSION

The data on the phytochemical constituents and distribution of carotenoids shows that the selected leafy vegetables are nutritionally and medicinally important.Leafy vegetables of Indian sources such as coriander, methi, curryleaves, spinach and broccoli are high in Chlorophyll, Xanthophylls and Lutein content whereas of Egyptian sources such as thyme, basil, oregano and marjoram are high in polyphenols and flavonoids.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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