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NUTRITIONAL ANALYSIS, TOTAL PHENOLIC CONTENT, FREE RADICAL SCAVENGING ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF LEAVES POWDER OF *MORINGA OLEIFERA* (DRUMSTICK*)* AND *CICER ARIETINUM* (CHICK PEA)

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ABSTRACT

This study aims to determine nutritional as well as medicinal properties of *Moringa oleifera* (Drumstick) and *Cicer arietinum* (Chick pea) leaves. The possible health benefits of these underutilized leafy vegetables were explored by determining total phenolic content by Folin-Ciocalteau reagent, free radical-scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl and phytochemicals extracted with acetone, chloroform, petroleum ether and ethanol by GC-MS screening. The *M. oleifera* and *C. arietinum* leaves powders were good source of protein, crude fiber, vitamin C and total minerals, particularly calcium, phosphorus and iron. Total phenolic content of drumstick and chick pea leaves was 260 mg/g and 190 mg/g, whereas free radical scavenging activity was 36.06 and 80.12 µg/ml respectively. Major phytochemicals identified in both leaves were hexadecanoic acid, pentacosane, β-sitosterol, phytol, octadecatrienoic acid methyl ester and vitamin E acetate. All the compounds identified by GC-MS were medicinally valuable for the treatment of various human ailments.

KEYWORDS: Drumstick leaves, Chick pea leaves,Total phenolic content, Free radical scavenging activity, Phytochemicals.

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INTRODUCTION

Nutritional deficiencies are mainly associated with poor quality and quantity of macro as well as micro nutrients from food. Beside this there has been an increase in the development of chronic diseases such as cancer, diabetes, cardiovascular disease, and hypertension in the last few years, which can be explained, at least in part, by factors such as diet and lifestyle[1]. Leafy vegetables are a rich source of healthy and important nutrients, as well as disease fighting constituents which have raised interest among scientists, food manufacturers, producers, and consumers for their roles in the maintenance of human health[2,4]. Drugs obtained from plants are believed to be much safer and exhibit a remarkable efficacy in the treatment of various ailments [5, 6]. Nutrients are essential for development and maintenance of good health. Along this, phytochemicals are bioactive substances of plants that have been associated in the protection of human health against diseases. Among phytochemicals, phenolics and Vitamin C have been shown antioxidant activity by inactivation of damaging free radicals[7].*Moringa oleifera* tree is known for its immature green pods called “Drumsticks” and *Cicer arietinum* plants is mainly cultivated for legume called “Chick pea”. The utilization of leaves of these plants as vegetable is very less and is mostly discarded or goes waste. The leaves are abundantly available. Hence, in the present study attempts have been made to prepare leaves powder of these underutilized leafy vegetables which will be used as quality supplement and to determine nutritional quality of it and explore the possible health benefits by determining total phenolic content, free radical-scavenging activities and phytochemicals.

MATERIALS AND METHODS

(i) Leaves collection and their preparation for drying

Fresh, green, un-damaged, non - insect infested leaves of *M. oleifera* and *C. arietinum* were procured from local farmers of Kolhapur, Maharashtra, India and the leaves were separated from stalk. The leaves were washed using potable water and were hung in an airy space to drain away extra water separately. These leaves were blanched in hot water (≈ 75^{o}C±1^{o}C) for retention of chlorophyll and to inactivate enzymes responsible for discoloration. The residual moisture was evaporated by spreading on a stainless steel tray at a room temperature for 30 minutes.

(ii) Preparation of leaves powder

The leaves were dried using tray drier, at 50^{o}C till they became crisp and brittle to touch and of moisture content ≈ 6%. The leaves took 5 hours for drying. The dried leaves were ground using lab scale grinder; the powder obtained was passed through 149 micron sieve.

(iii) Nutritional analysis

The prepared powder samples were evaluated for their nutritional quality with respect to moisture, protein, fat, ash, crude fiber, total carbohydrates, calcium, iron, phosphorous and vitamin C[8].

(iv) Total phenolic content estimation

The powdered samples of leaves were subjected to extraction (100 g each) by maceration in methanol (500 ml) and were kept at room temperature in the dark for 10 days with occasional shaking. The macerates were filtered, and the filtrates were dried at low temperature (45°C) under vacuum in a rotary evaporator. The extracts were kept in dark at 4°C for further analysis. The total phenolic contents of plant samples were determined with Folin-Ciocalteau reagent using the UV/Visible spectrophotometer. The results were expressed as milligram of gallic acid equivalents per gram (mg/g) of the dry extract[7].

(v) DPPH radical scavenging activity

Antioxidant activity for methanol extract of leaves was evaluated, by employing radical scavenging assays; 2, 2 -diphenyl, 1-picryl

hydrazyl (DPPH). The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm. EC50 value is the extract concentration at which DPPH radicals were reduced by 50% and calculated from the linear regression analysis from the obtained radical scavenging activity values[7].

(vi) Phytochemical Analysis by GCMS

The Plant Extracts was prepared by taking 2 g of leaves powder in stopper reagent bottle and mixed with 20 ml of solvent (Acetone, Chloroform, Petroleum ether and Ethanol) and kept for 12 hours in the dark, at room temperature (≈30°C±2°C) with intermediate shaking. The mixture was then filtered through Whatman's filter paper no. 1 and the filtrate was collected in a beaker. The extract was concentrated to 5ml, by placing them open at room temperature[9].The screening of phytochemicals was carried out by taking 1 ml concentrated extract on Gas Chromatography Mass Spectroscopy (GC-MS - Model; QP 2010 series, Shimadzu, Tokyo, Japan) equipped with Silica capillary column of 30 m length, 0.25mm diameter and 0.25µm film thickness. For GC detection an electron ionization system with ionization energy 50eV was used. Helium (99.99%) gas was used as carrier gas at a linear velocity 36.3cm/sec. The total flow, column flow and purge flow were 74.1ml/min, 1ml/sec and 3.0ml/min. respectively, at a pressure 53.6KPa. The column oven temperature and injector temperature were set at 50.0°C and 200.0°C respectively. 1µl of respective sample was injected manually in split mode, with split ratio of 1:70. In MS analysis ion source temperature and interface temperature were 200.0°C and 280.0°C respectively, with solvent cut time 5.5min. The detector voltage was 1.5 kV. The relative area of each extract constituent was expressed as percentage with peak area normalization. The identification of compounds was assigned by comparison of their retention indices and mass spectra fragmentation pattern obtained in GCMS chromatogram with those stored in computer libraries and also with published literature.

RESULTS AND DISCUSSION

1. Nutritional analysis

Table 1
Nutritional composition of Moringa oleifera Leaves And Cicer arietinum Leaves(per 100g powder).

Parameters	*Moringa oleifera* Leaves*	*Cicer arietinum* Leaves*
Moisture (g)	6.20 ± 0.26	5.97 ± 0.54
Protein (g)	23.78 ± 0.75	24.84 ± 0.12
Fat (g)	6.58 ± 0.10	5.30 ± 0.34
Ash (g)	9.54 ± 0.08	7.89 ± 0.06
Crude Fiber (g)	8.21 ± 0.33	11.37 ± 0.51
Total Carbohydrate (g)	53.90 ± 0.12	56.00 ± 0.15
Calcium (g)	3.465 ± 0.09	2.679 ± 0.08
Iron (mg)	19 ± 0.06	514 ± 0.05
Phosphorus (mg)	206 ± 0.02	368 ± 0.04
Vitamin C (mg)	59 ± 0.11	23 ± 0.08

(*The values are mean ± S.D. of three determinations)

The nutritional analysis of prepared leaves powder reveals that both the leafy vegetables are excellent source of macro as well as micro nutrients (Table 1). The protein content was 23.78% and 24.84% in *M. oleifera* and *C. arietinum* respectively. These values denote that the protein content of these powder samples is somewhat same to that of legumes[10]. The protein content may serve as solution to combat protein efficiency

malnutrition. The amount of fiber was 8.21% and 11.37% in *M. oleifera* and *C. arietinum* respectively. The fiber content of *C. arietinum* was high as compared to *M. oleifera*. The high amount of fiber will exert a positive effect against constipation by its ability to absorb water[11]. The common leafy vegetables are known for their high amount of mineral and vitamin content and proven as a potential source for micro nutrients. The results of ash content showed in table 1 reveals that the studied leaves are also a best source of total minerals. Further, the minerals of great importance for human health that is calcium and iron are also present in very good amount. Among the two leaves *M. oleifera* contains higher amount of calcium and *C. arietinum* contains very high amount of iron. The good amount of calcium and iron may help in reducing diseases like osteoporosis and anemia[10]. The phosphorus content was also good. The vitamin C content was 56 mg and 23 mg per 100g powder of *M. oleifera* and *C. arietinum* respectively. Hence, the prepared powder may act as pool of essential nutrients and can become a quality supplement in various health food formulations.

2. Total phenolic content estimation

Table 2
Total phenolic content and DPPH scavenging activity (EC50 value) Of Moringa oleifera and Cicer arietinum (Dry extracts)

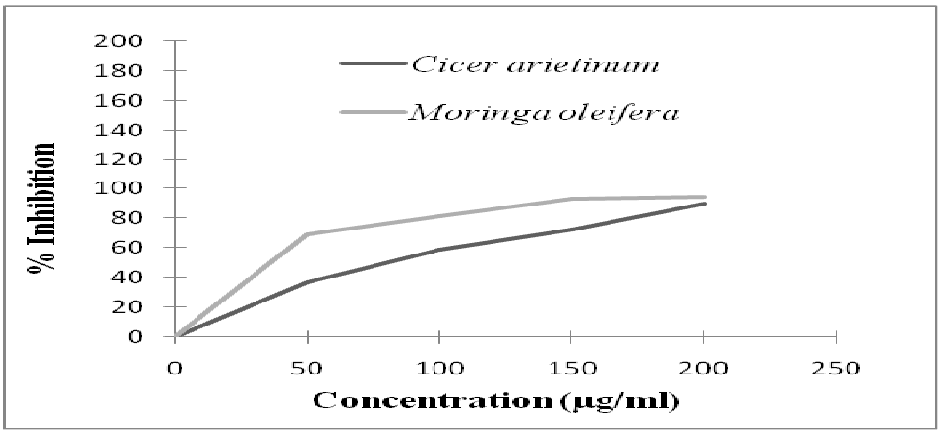
Sample	Total Phenolic Content* (mg/g)	EC50 Value µg/ml*
Moringa oleifera Leaves	260 ± 2.6	36.06 ± 0.6
Cicer arietinum Leaves	190 ± 1.8	80.12 ± 0.8

(*The results are given as mean ± S. D. of three determinations)

The total phenolic content of *M. oleifera* and *C. arietinum* leaves were 260 mg and 190 mg of gallic acid equivalent per gram of dry extract. The obtained values denote high phenolic content. Since it is reported earlier that high phenolic content are responsible for the antioxidant activity[12,14], the obtained amount of total phenolic content in the extract indicated that the extract possess a high antioxidant activity.

3. DPPH radical scavenging activity

Figure 1
DPPH free radical scavenging activity of methanolic extract of Moringa oleifera and Cicer arietinum.



The results obtained for % inhibition of DPPH are shown in figure 1. It indicates that as the concentration of extract increases the inhibition of DPPH get also increased. For *M. oleifera* extract the activity was constant after 100µg/ml concentration. This shows that at a very little concentration *M. oleifera* extract is active. The results of DPPH reduction by methanolic extracts of plants are expressed as EC50 values and shown in table 2. A low EC50 value is the sign of strong antioxidant activity. The EC50 values of DPPH scavenging activity were 36.06 and 80.12µg/ml for *M. oleifera and C. arietinum* respectively. Among them *M. oleifera* posses high antioxidant activity compared to *C. arietinum*. The EC50 value was lower than 100µg/ml of both samples indicates high scavenging potential, which can be correlated with high total phenolic content in leaves extract. Free radicals are highly reactive chemical substances, which can lead to accelerated aging, cellular injuries, cancers, cardiovascular diseases and inflammations etc. In the presence of antioxidant molecule free radicals (DPPH) get reduced, giving rise to discolored solution. The degree of discoloration indicates the scavenging potential of the antioxidant extract, which is due to the radical scavenging ability. It has already been exhibited that phenolic compounds are responsible for radical scavenging activity, due to the ease of their hydrogen atom donation to active free radical[12, 14].

4. Phytochemical Analysis by GCMS

The chemical constituents, identified by comparison of their retention indices and mass spectra fragmentation pattern obtained in GCMS chromatogram (Figure 2, 3, 4, 5, 6, 7, 8, and 9) with those stored in computer libraries and with published literature[9, 16 and 17], were enumerated along with molecular formula, retention time, molecular weight and peak area in table 3 and 4 The results for *M. oleifera* from the peak area showed that, more components were extracted in petroleum ether. The major components present in acetone extract were pentacosane (34.46%), heptacosane (15.99%) and phytol (10.65%), and in chloroform extract pentacosane (51.83%), heneicosane (12.93%), vitamine E acetate (10.30%) and phytol (8.14%). The petroleum extract components were pentacosane (24.74%), 9,5,11 octadecatrienoic acid, methyl ester (21.90%), docosane (21.04%), phytol (11.32%) and Nonacosanol (9.13%). The ethanol extract showed that it contains tetradecanal (31.21%), vitamin E acetate (23.04%), pentacosane (19.08%) and nonacosanal (12.79%). Also, the results for *C. arietinum* from the peak area showed that, more components were extracted in chloroform. The acetone extract contains 1,3,12, nonadecatriene-5, 14-diol (22.77%), hexadecanoic acid (20.63%), 3,7,11,15, tetramethyl-2-hexadecen-1-ol (8.53%) and octadecanoic acid (8.22%). In chloroform extract, 9,5,11 octadecatrienoic acid, methyl ester (25.42%), tetracosane (19.53%), vitamin e acetate (11.99%) and phenol, 2, 5-bis [1,1-dimethyl ethyl] (13.20%). The results of petroleum ether and alcohol extract showed very less component and with very less concentration. The obtained results of petroleum ether extract identified vitamin E acetate (4.86%) and 9, 12, 15 octadecatrienoic acid methyl ester (2.54%), and that of ethanol extract identified 9, 12, 15 octadecatrienoic acid methyl ester (7.92%), hexadecanoic acid (6.02%) and 9H – fluorene, 9 phenyl (5.29%). Also, the literature has stated that identified components posse activities like antioxidant, antimicrobial, anticancer, antitumor, anti-proliferative, anti-inflammatory and hypolipidemic activity[18, 19 and 20].

Figure 2
GCMS Chromatogram of Acetone Extract of Moringa oleifera leaves powder

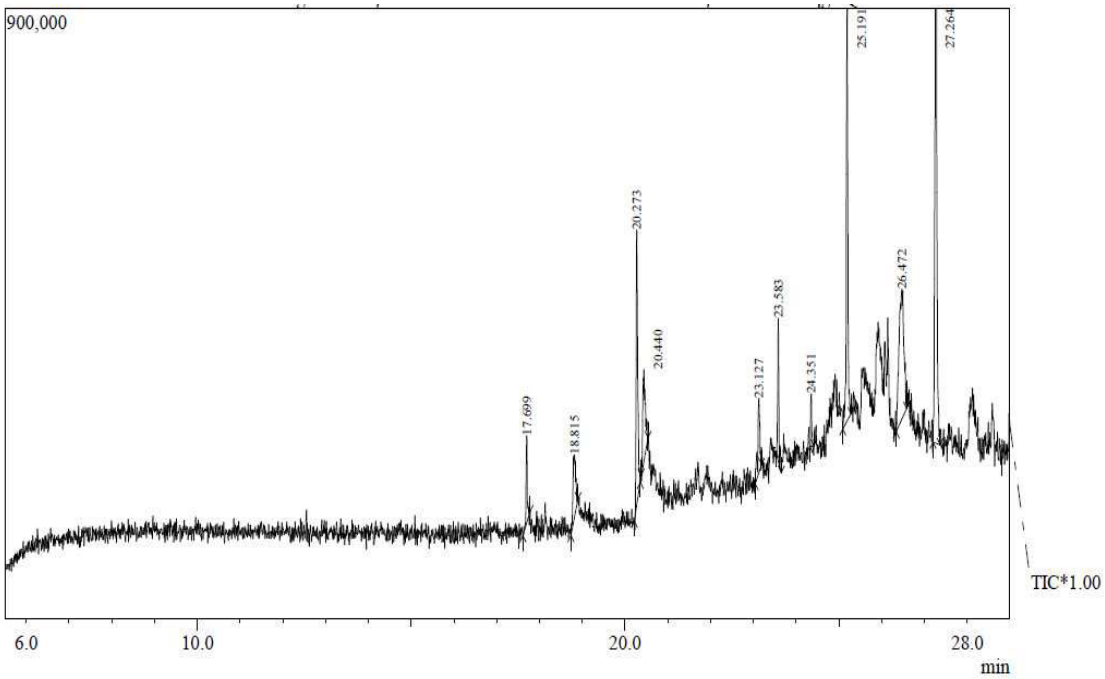


Figure 3
GCMS Chromatogram of Chloroform Extract of Moringa oleifera leaves powder

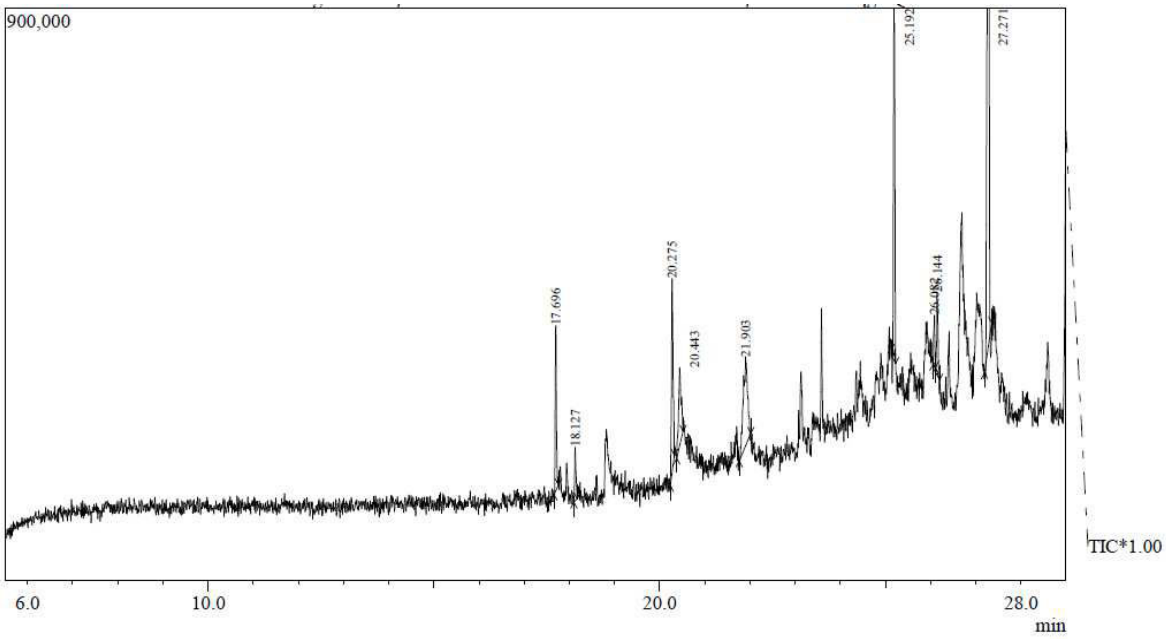


Figure 4
GCMS Chromatogram of Petroleum Ether Extract of Moringa oleifera leaves powder

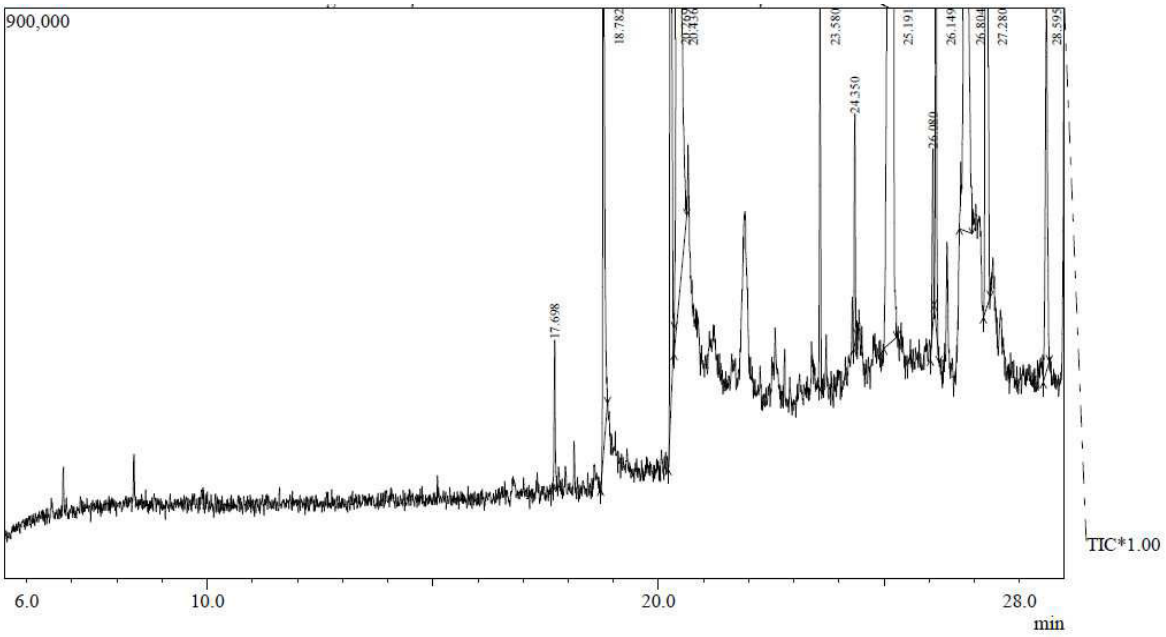


Figure 5
GCMS Chromatogram of Ethyl Alcohol Extract of Moringa oleifera leaves powder

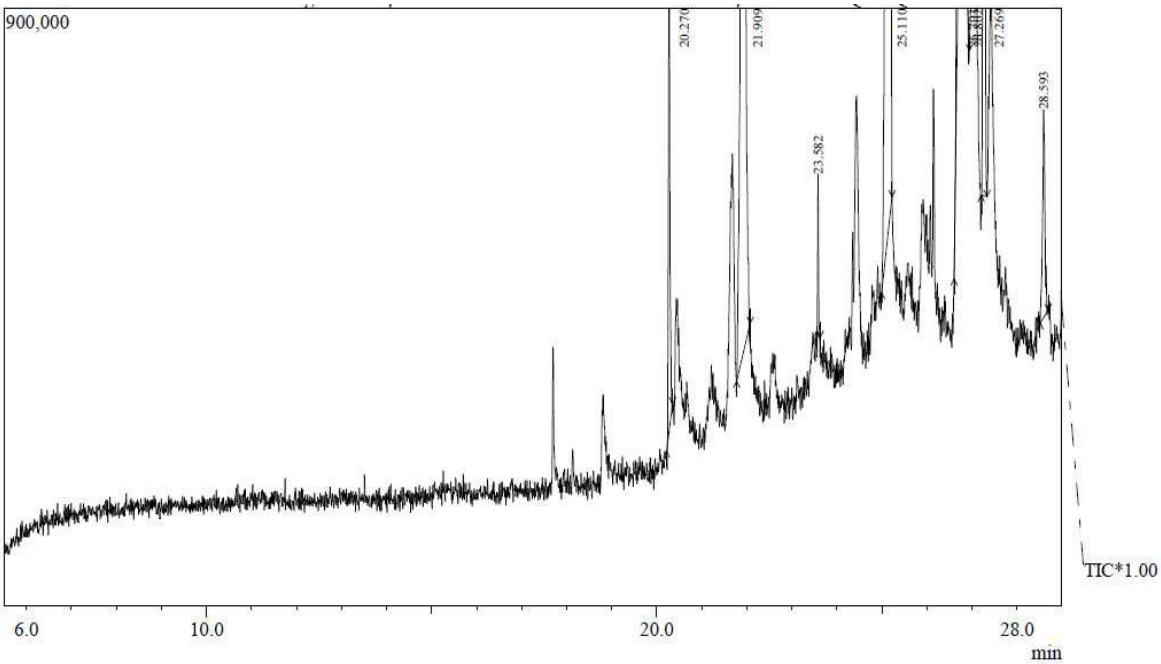


Figure 6
GCMS Chromatogram of Acetone Extract of Cicer arietinum leaves powder

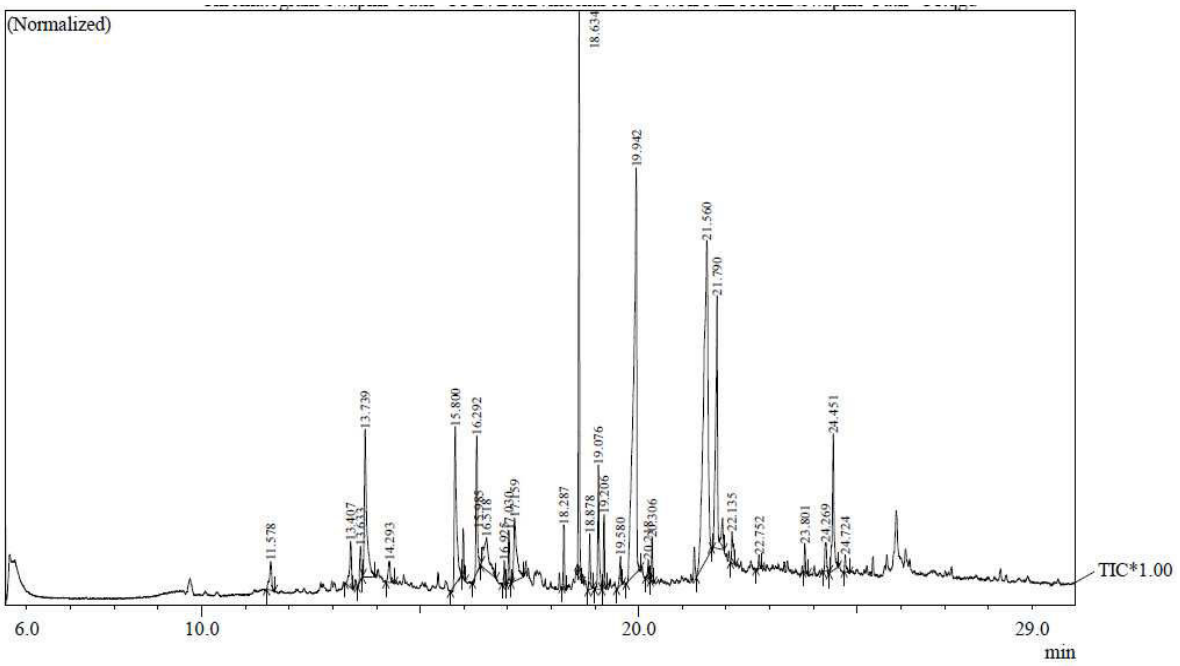


Figure 7
GCMS Chromatogram of Chloroform Extract of Cicer arietinum leaves powder

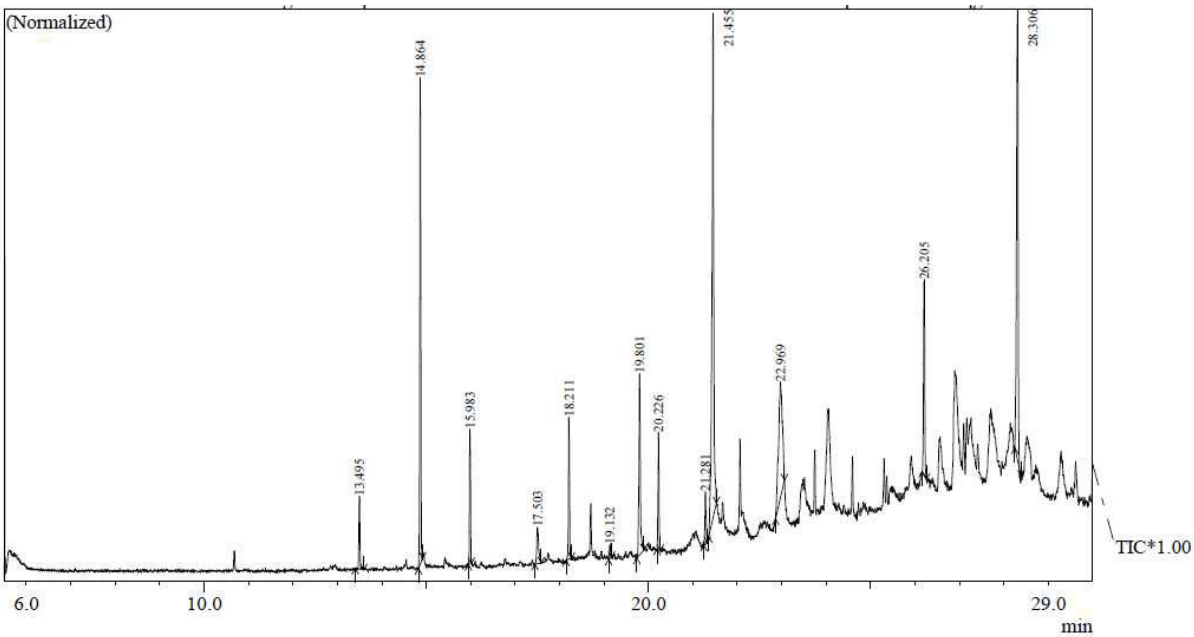


Figure 8
GCMS Chromatogram of Petroleum Ether Extract of Cicer arietinum leaves powder

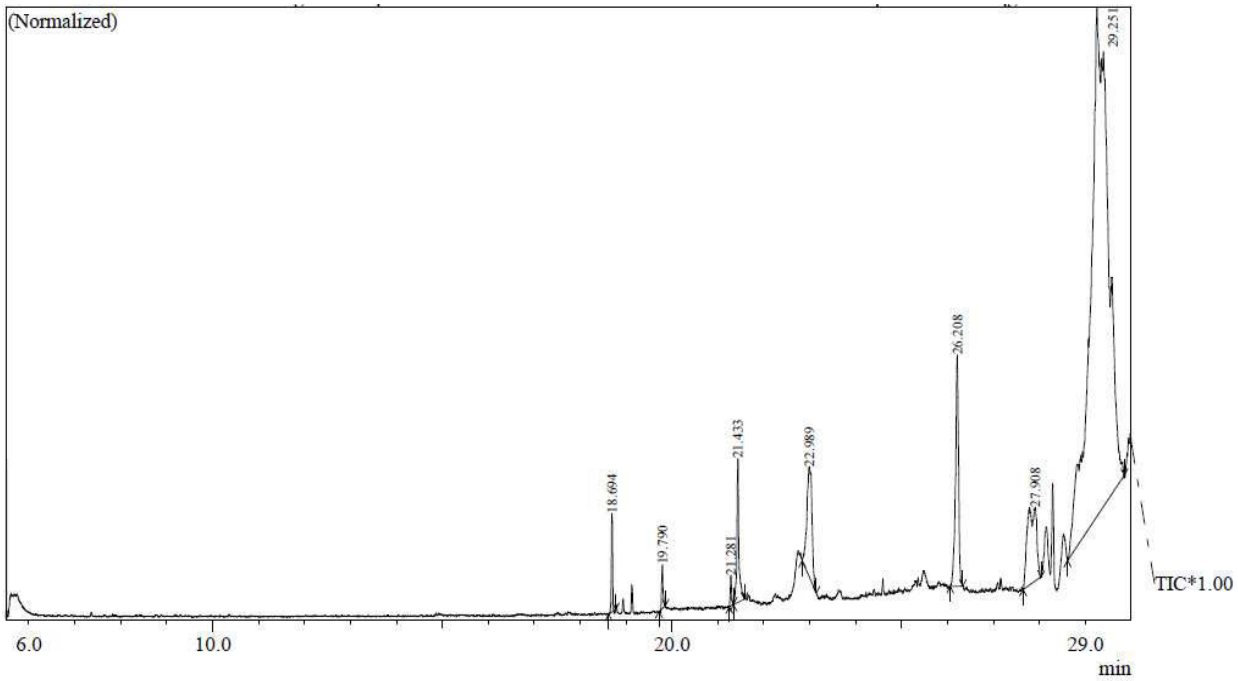


Figure 9
GCMS Chromatogram of Ethyl Alcohol Extract of Cicer arietinum leaves powder

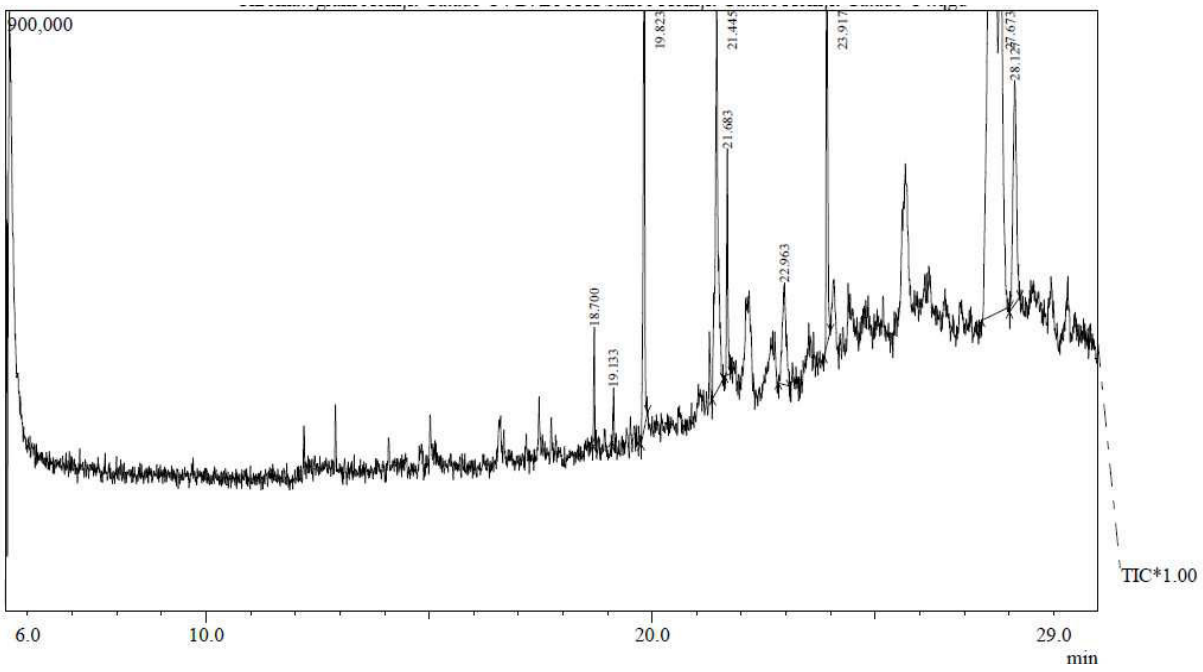


Table 3
Major phytoconstituents screened by GC-MS in M. oleifera leaves powder extract.

No	RT*	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
I - Acetone Extract					
1	27.264	Pentacosane	$C_{25}H_{52}$	352	34.46
2	25.191	Heptacosane	$C_{27}H_{56}$	380	15.99
3	23.583	Heptadecane, 2 Methyl	$C_{18}H_{38}$	254	4.24
4	23.127	Carbamic acid, 2(dimethyl amino) ethyl ester	$C_5H_{12}N_2O_2$	129	3.93
5	20.273	Phytol	$C_{20}H_{40}O$	296	10.65
6	17.699	1- Hexadecyne	$C_{16}H_{30}$	222	2.54
II - Chloroform Extract					
1	27.271	Pentacosane	$C_{25}H_{52}$	352	51.83
2	25.192	Heneicosane	$C_{21}H_{44}$	296	12.93
3	21.903	Vitamin E acetate	$C_{31}H_{52}O_3$	472	10.30
4	20.275	Phytol	$C_{20}H_{40}O$	296	8.14
5	17.696	Z-4 Octadecen-1-ol acetate	$C_{20}H_{38}O_2$	310	5.57
III - Petroleum Ether Extract					
1	27.280	Pentacosane	$C_{25}H_{52}$	352	24.74
2	26.804	Nonacosanol	$C_{29}H_{60}O$	424	9.13
3	25.191	Docosane	$C_{22}H_{46}$	310	21.04
4	23.580	Heptadecane, 2 Methyl	$C_{18}H_{38}$	254	1.52
5	20.436	9,5,11- Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	21.90
6	20.269	Phytol	$C_{20}H_{40}O$	296	11.32
7	18.782	Octadecanoic acid, 2-(2-hydroxy ethoxy) ethyl ester	$C_{22}H_{44}O_4$	372	4.28
IV - Ethyl Alcohol Extract					
1	27.269	Pentacosane	$C_{25}H_{52}$	352	19.88
2	26.802	Nonacosanol	$C_{29}H_{60}O$	466	12.79
3	26.701	β – Sitosterol	$C_{29}H_{50}O$	414	4.56
4	25.110	Tetradecanal	$C_{14}H_{28}O$	212	31.21
5	21.909	Vitamine E acetate	$C_{31}H_{52}O_3$	472	23.04
6	20.270	Phytol	$C_{20}H_{40}O$	296	4.55

(*RT= Retention Time)

Table 4
Major phytoconstituents screened by GC-MS in Cicer arietinum leaves powder extract.

No	RT*	Name of the Compound	Molecular Formula	Molecular Weight	Peak Area (%)
I - Acetone Extract					
1	21.790	Octadecanoic acid	$C_{18}H_{36}O_2$	284	8.22
2	21.560	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	22.77
3	19.942	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.63
4	19.583	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.65
5	18.634	3,7,11,15 – Tetramethyl -2- hexadecen -1-ol	$C_{20}H_{40}O$	296	8.53
6	15.800	1(2H) – Naphthalenone,3,4- dihydro -6- methoxy	$C_{11}H_{12}O_2$	176	5.25
7	13.739	Phenol, 2,5, bis (1,1- dimethylethyl)	$C_{14}H_{22}O$	206	5.71
II - Chloroform Extract					
1	28.306	Tetracosane	$C_{24}H_{50}$	338	19.53
2	22.969	Vitamin E acetate	$C_{29}H_{50}O_2$	430	11.99
3	21.455	9,12,15 – Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	25.42
4	21.283	Phytol	$C_{20}H_{40}O$	296	1.68
5	20.226	1-Octadecanol	$C_{18}H_{38}O$	270	3.10
6	19.801	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.92
7	18.211	Octadecene	$C_{18}H_{36}$	252	3.87
8	15.983	Hexadecene	$C_{16}H_{32}$	224	3.55
9	14.864	Phenol, 2,5, bis (1,1- dimethylethyl)	$C_{14}H_{22}O$	206	13.20
III - Petroleum Ether Extract					
1	26.208	1, 30 – Tetracontanediol	----	----	6.05
2	22.989	Vitamin E acetate	$C_{29}H_{50}O_2$	430	4.86
3	21.433	9,12,15 – Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	2.54

4	21.283	Phytol	$C_{20}H_{40}O$	296	0.33
5	19.790	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	0.49
6	18.694	3,7,11,15 – Tetramethyl -2- hexadecen -1-ol	$C_{20}H_{40}O$	296	1.00
IV - Ethyl Alcohol Extract					
2	23.917	9H – Fluorene, 9 Phenyl	$C_{19}H_{14}$	242	5.29
3	22.963	Vitamin E acetate	$C_{29}H_{50}O_2$	430	2.77
4	21.683	Octadecanoic acid	$C_{18}H_{36}O_2$	284	1.97
5	21.445	9,12,15 – Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	7.95
6	19.823	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.02
7	18.700	3,7,11,15 – Tetramethyl -2- hexadecen -1-ol	$C_{20}H_{40}O$	296	0.72

(*RT= Retention Time)

CONCLUSION

Findings of the study indicate that the studied plants (leafy vegetables) are excellent sources of macro as well as micro-nutrients which will be helpful in combating nutritional deficiencies like Kwashiorkor, Osteoporosis, Anemia and Cardiovascular diseases. Their fiber content provides bulk in the diet and this helps to enhances gastrointestinal function, prevents constipation and may reduce cholesterol content. It also bears a potent antioxidant activity. The constituents scavenge free radicals and exert a protective effect against oxidative damage. The antioxidant potential may be attributed to the presence of polyphenolic compounds. The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status. The presence of the identified phytochemicals makes the leaves pharmacologically active as they are reported to have potential like antimicrobial, anticancer, antioxidant, anti-inflammatory and hypochlolesteromic activity. The consumption of these underutilizes, cheep and readily available vegetables powder may have many beneficial health attributes and may become a quality supplement.

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