ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

Phytochemical screening of anti-aging medicinal plants of Celery (*Apium graveolens*), Safflower (*Carthamus tinctorious*) and Pomegranate (*Punica granatum*) aqueous extracts

Zena M Qaragholi[1], Ahmed Rahmah Abu-Raghif[2], Enas Jawad Kadhim[3]

Lecturer-Department of pharmacognosy and medicinal plants-College of pharmacy- University of Baghdad[1]
Prof. Department of pharmacology- College of medicine- University of Nahrain[2]
Prof. Department of pharmacognosy and medicinal plants-College of pharmacy- University of Baghdad[3]

ABSTRACT— Preliminary literature review on Baghdad's aging population and the authors' experiences have demonstrated that the challenges associated with aging process have not been given much attention. Anti-aging plants which have been associated with health promoting properties have been utilized as interventions to curb the aging process. This has been achieved as they contain active ingredients which have been scientifically proven to have medicinal and antiaging properties through various laboratory species as previously reported. This study sought to identify these phytochemicals both quantitatively and qualitatively using different techniques. Three plants, *Apium graveolens, Carthamus tinctorious* and *Punica granatum* were obtained at a local market in Baghdad and extracted with water. The resultant mixture was purified by a whatman paper and the solvent dried using a spray dryer. The resultant residue was kept for further analysis. Qualitative method of screening different phytochemicals among them tannins, carbohydrates, glycosides, phenols, resins, flavonoids, saponins, alkaloids, proteins, coumarins, terpenes and steroids was done. The methanolic extracts were further screened using GC-MS and their mass spectrum compared with compounds from the database library. Separation and identification of the three extracts was also done using High Performance Liquid Chromatography and mineral analysis of selenium, zinc and calcium were conducted using Atomic Absorption Spectroscopy. GC-MS results revealed that Safflower had the highest number of major compounds while celery plant had the least. In safflower Triisobutyl (3-phenylpropoxy) silane was the highest in concentration at 16.73% while in celery plant the highest active component was 1H-Indole at a concentration of 16.37%. In pomegranate plant, the highest active component was 6-Octadecenoic acid, methyl ester with a concentration of 20.24%. HPLC analysis did not reveal the six standards used in this study for safflower and pomegranate plants however, in celery plant resverastrol, b-sistine and rutin were identified at concentrations of 4.93%, 16.09% and 5.22 % respectively. Qualitative phytochemical screening showed that safflower and pomegranate exhibited similar trend in all the phytochemicals and also there presence was more compared to celery plant. Selenium ion was not detected although zinc was found but in minute concentrations. Calcium ion was also detected in high concentrations although it was significantly lower in pomegranate plant at 35.4%. In conclusion, GC-MS profiling of the phytochemicals among the three plants revealed varying components with different concentrations. This was also reflected on the qualitative determinations although pomegranate and safflower exhibited similar occurrence of the phytochemicals.

KEYWORDS: anti-aging medicinal plants, aqueous extracts, Celery

1. INTRODUCTION

Phytochemical are plant non-nutrient active components found virtually in all plants. They are associated with decreased risk of chronic and age-related deteriorating ailments [27]. Aging is considered as an active and complex biological process involving many players which is as a result of genetic and environmental factors [20]. Several theories have been drafted regarding the aging process with the most accepted one being that of free radical theory by Professor Harman in 1956 [34]. Free radicals formation has led to oxidative damage which has been attributed to cellular dysfunctions and physiological decline leading to aging, characterized by degenerative diseases and eventually death [5]. This complexity of the idea of aging and the associated age-related health challenges especially the social effects associated with Human Immno Virus and AIDS has presented a major challenge to the aging generation and medical fraternity of Iraq [34]. Preliminary literature review on Baghdad's aging population and the author's experiences have demonstrated that the challenges associated with aging process have not been given much attention [16]. Anti-aging plants which have been associated with health promoting properties have been utilized as interventions to curb the aging process. This has been achieved as they contain active ingredients which have been scientifically proven to have medicinal properties. In the present study three know medicinal plants were evaluated for their qualitative and quantitative phytochemical components. The three plants were Celery (*Apium graveolens*), *Safflower* (*Carthamus tinctorious*) and Pomegranate (*Punica granatum*) extracts.

Celery, *Apium graveolens L*. is widely used as a herb as well as a medicinal plant globally since ancient times [23]. The plant is utilized for its seeds, leaves and essential oils. Some of its phytochemicals, constituting of carbohydrates, flavonoids, alkaloids and steroids have been reported [48]. The seeds are utilized as spice because of their palatable taste and aromatic odor. In ancient medicine it is used as a diuretic, or brewed for relieving hypertension, bronchial asthma, liver and spleen ailments [6]. Investigations on its chemical constituents have resulted in characterization of several flavonoids among them luteolin, hesperitin, quercitin and apigenin conjugates as well as other phenolic and cinnamic acid derivatives [43]. More so, the characteristic smell of the celery plant has been attributed to the lactone sedanolide (3-butyl-3a, 4, 5, 6-tetrahydrophthalide), sedanolide and other related phthalates [10]. Coumarins of varying classes, mostly furano coumarins, have also been reported [8]. Because of its richness and different contents of phenolic compounds as well as minerals and vitamins, this plant has been attributed with antimicrobial and antioxidant activities, and a combination of other attributes associated with pharmacology [24].

On the other hand safflower, *Carthamus tinctorius L*., is cultivated in semi-arid regions chiefly for its seed that exhibits a high level oil content. The oil known to possess saturated and unsaturated fatty acids coupled by its tocopherol content [33]. The flowers have been historically utilized as colorants in foods as well as dye in the clothing sector. In addition, the bioactive compounds such as polyphenols have been attributed to its colour and taste [9]. [25], [36] have also reported the phenolic compounds and antioxidant activity associated with its seeds and petals. [36] investigated its phytochemicals and found that gallic acid was the most predominant in its flowers. Other phenolic compounds found included epicatechin, syringic acid, chlorogenic acid and quercetin-3-galactoside. In addition, quinochalcone C-glycosides have also been reported in safflower with the most predominant been the water soluble components of hydroxy safflor yellow A, safflor yellow A, and hydroxy safflor yellow B [22]. Bioactive properties of hydroxy safflor yellow A have been utilized in Chinese medicine for the treatment of cerebrovascular and cardiovascular disease [46], [47].

Lastly, the pomegranate plant is a native crop crossing from Iran to the Himalayas in north India [4]. It has

been cultivated since ancient times across the Mediterranean regions of Asia, Africa and Europe [40]. Its chemical composition can differ depending on climate, maturity, region, cultivation practices, and storage conditions among others [4]. Approximately 50% of the fruit weight is contained in the peel, which is rich in ellagitannins, phenolics, flavonoids as well as proanthocyanidin components [12]. These compounds comprise the major antioxidants which are important as a result of their free radical scavenging action. It is rich in minerals including phosphorus, potassium, magnesium, nitrogen, calcium and sodium among other complex polysaccharides. The edible part of the fruit consists of 40% arils and 10% seeds. It's also comprised of organic acids, such as citric acid, ascorbic acid and malic acid, among other phenolics and flavonoid constituents [1]. Pomegranate juice contains antioxidants such as polyphenols and punicalagins at higher levels than most other fruit juices [2]. In previous studies, ellagitannins and hydrolyzable tannins have been detected and quantified in pomegranate juices which accounts for the high antioxidant activity of the fruit [2]. More so, the plant gained popularity globally because of its high nutritional value [19] and its active ingredients which possess medicinal properties associated with its juice [31].

From the above review it is evident that the three selected plants have medicinal as well as anti-aging properties attributed to their phytochemical components. Therefore this study sought to identify and profile the major components of these bioactive ingredients as well as some selected minerals.

2. MATERIALS AND METHODS

2.1 Plant extraction

The three plants (*Apium graveolens*, *Carthamus tinctorious* and *Punica granatum*) were collected at a local market in Baghdad, washed with tap water, air-dried in the shade, ground and extracted with water. Maceration technique was used using a shaking incubator Si-600R. The air dried herbs were grinded using a mechanical grinder then mixed with water. The water solution was incubated for two days and the extract purified with a whatman paper. The solvent was dried using a spray dryer, Bochi Mrs B-200, and the residue kept for further analysis [14].

2.2 Qualitative method of phytochemical screening

Different phytochemical determinations were performed using the three obtained extracts. The procedures have been previously reported by several authors. They were used without any modifications [18], [39], [42], [30], [29], [17].

2.3 Phenols

To test phenol presence, 1ml of the extract and 2 ml of distilled water were added in a test tube followed by few drops of 10% ferric chloride. Appearance of a blue or green color indicated a positive test of phenols.

2.4 Flavonoids

To test for flavonoids, 1 ml of the extract and a few drops of dilute sodium hydroxide were put in a test tube. An intense yellow color which becomes colorless on addition of a few drops of dilute acid is a positive test for flavonoids.

2.5 Tannins

To test for tannins, 1 ml of 5% ferric chloride was added to solvent free extract in a test tube. The presence of tannin is indicated by the formation of a bluish black or greenish black precipitate.

2.6 Saponins

To test for saponins, the extract was diluted with 20 ml distilled water and agitated in a graduated cylinder for 15 minutes. The formation of 1cm layer of foam was a positive test for saponins.

2.7 Cardiac Glycosides

To test the cardiac glycoside presence, in a test tube 5 ml of extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Afterwards it was underplayed with 1 ml concentrated sulphuric acid. A brown ring at the interface indicates a de-oxy sugar characteristic of cardenolites.

2.8 Steroids

To test for steroids, 1 ml of extract was dissolved in 10 ml chloroform and equal volume of concentrated sulphuric acid added by the sides of a test tube. When the upper layer turns red and sulphuric acid phase shows yellow with green fluorescence is an indication of the presence of steroids.

2.9 Terpenoids

To test the presence of terpenoids, 5 ml of each extract was mixed with 2 ml of chloroform in a test tube and 3ml of concentrated sulphuric acid added to form a layer. Formation of a reddish brown precipitate at the interface indicated the presence of terpenoids.

2.10 Coumarins

To test the presence of coumarins, 2ml of ammonia solution were added to 5ml of extract of each plant. The formation of a reddish color confirmed the presence of coumarins.

2.11 Protein

To test the presence of proteins, 1 ml of each extract was added in test tubes add 0.2 ml of 1 M NaOH solution added to make it alkaline. Finally 200 µl of Biuret reagent was added to the mxture. The formation of a violet colored product indicated a positive test for proteins.

2.12 Alkaloids

1ml of the extract was stirred with 5ml of 1% aqueous HCl in a steam bath and filtered while hot. Distilled water was added to the residue and 1ml of the filtrate treated with few drops of Wagner's reagent (solution of iodine in Potassium iodide). The formation of a reddish-brown precipitate is a positive indication for alkaloids.

2.13 Resins

Deposition of a red precipitate when 2ml of extract of each plant samples was boiled with 1% aqueous hydrochloric acid was taken as evidence for the presence of resins.

2.14 Carbohydrates

2ml of the sample was put in a dry test tube and 2-3 drops of Molisch's reagent added to the solution. Gently, 1ml conc. H_2SO_4 was pipetted along the sides of the tube to form two distinct layers. Appearance of purple color at the interface indicates the presence of carbohydrates.

2.15 Phytochemical screening using Gas chromatography coupled with MS detector

The methanolic extract of the three extracts were identified using Shimadzu GC-2010 Gas chromatography coupled with QP2010 mass spectrometer. The extracts were injected into the GC-MS fitted with a 30m glass capillary column with a film thickness of 0.25 µm. Helium gas was used as a carrier at a constant flow rate of 1ml/min. Injector and detector temperatures were both maintained at 250°C. The GC temperature

was programmed within the range of 60°C-280°C at 15°C/min and a split ratio of 1:30. The total GC run time was set at 35 min. The MS scan parameters included a mass range of m/z 40-1000 at a scan interval of 0.5secs with a scan speed of 1000 amu/sec. Compound identification was conducted using the database of NIST08, WILEY8 and FAME Libraries. The mass spectrum of the individual unknown compounds was compared with the known compounds stored in the software database Libraries. The relative percentage amount of each component was calculated by comparing its peak area to the total corrected areas as previously reported [13].

2.16 Phytochemical screening using High-Performance Liquid Chromatography

Separation and identification of Pomegranate, Celery and Safflower extracts was done using High Performance Liquid Chromatography (HPLC). The water extracts for HPLC analysis were passed through a 0.45 μm filter (Millipore, MSI, Westboro, MA) before injection into a HPLC column C18. The mobile phase used was methanol mixed with 1% acetic acid. Kacefel, Silybinin, Resveratrol, Quercitin, B-Sistine and Rutin were used as the analytical standards to determine the unknown active components in the three extracts [28].

2.17 Mineral determination using atomic absorption spectroscopy

Dried and milled samples (0.50 ± 0.01 g) from the three extracts were placed in a porcelain dish of approximately 15 mL in volume. The samples were then ashed in a muffle furnace at 550°C for ~8 hr. The cold ash was dissolved in 3 mL concentrated HCl, and the solution diluted with 2% HCl (v/v) to a final concentration of 0.1N. The solution of acidified ash was filtered through filter paper, and the consecutive rinses [with 2% HCl (v/v)] from crucibles were collected in a 50mL volumetric flask. The samples were concentrated by dissolving the ash obtained after incineration of 0.50 g of sample in a final volume of 5 mL in order to obtain a sensitivity within the optimum working range specified for the AAS. The samples were analyzed by atomic absorption spectrometer with flame atomisation (Model 2280 Perkin Elmer, Spain). The measurements were made in hold mode with air- acetylene flame, where the air (as oxidant) was maintained at a flow rate of 50 mL min^{-1} and the acetylene (as fuel) maintained at a flow of 20 mL min–1, to attain a flame temperature of 2600°C. The hollow-cathode lamps were specific for each element analysed. To achieve maximum sensitivity and precision, the equipment was equilibrated by alignment of the lamp and adjustment of the selected wave- length.

3. RESULTS AND DISCUSSION

The authenticity of a given plant species is an important parameter to put into consideration [26] and should be determined before any biological evaluation or the utilization of a plant extract for medical as well as pharmaceutical applications are put into consideration. Several genetic as well as environmental attributes might affect the profile of these phytochemicals of a given plant extract thus influencing its biological activity [3]. This observation should also apply for the extraction, conditions of extraction and more specifically on the selection of the solvent being used for the extraction as they pose a significant influence on the phytochemical profile of these extracts [21]. Phytochemical characterization of these plant extracts is important in order to achieve results which are reproducible, but its composition is mostly interfered with because of the choice of the analytical method whether HPLC, UV-visible absorption, GC-MS or with other detectors) and thus its resolution [45]. Identification of the bioactive compounds is important but it also represents a challenge that such observed phytochemical activity could be the result of a complex synergism among varying compounds from different extracts thus making it a challenge during the process of identification [41]. Biological determination should be conducted in vitro to allow simultaneous evaluation of different plant extracts prior to their evaluation with laboratory animal models, and also before conducting comprehensive toxicity evaluation, epidemiological studies or even clinical trials [21], [38].

Figure 1 illustrates GC-MS chromatograms for safflower plant.

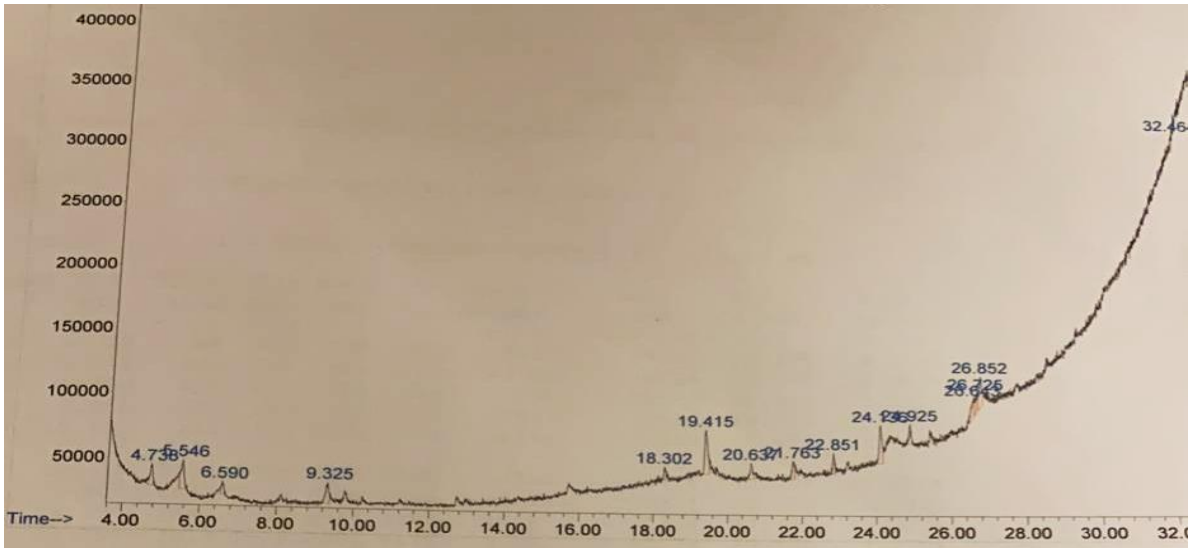

Figure 1: GC-MS chromatograms for safflower plant

The three medicinal plants exhibited varying metabolites as indicated in the respective tables. Safflower had the highest number of major compounds while celery had the least.

In safflower Triisobutyl (3-phenylpropoxy) silane was the highest in concentration at 16.73% followed by N-(3-Allyl-2-oxo-2, 3-dihydro-1, 3-benzothiazol-6-yl) acetamide at 13.04% as shown in table 1. The lowest active ingredient was for Purin-2, 6-dione at 4.88%. Previous studies have demonstrated that these active ingredients in safflower contribute in slowing hydrogen peroxide against injury to the body cells and organs and also neutralizing lipid peroxides as well as eliminating free radicals [44].

Table 1: Phytochemical screening for safflower plant using GC-MS

Peak No.	RT (min)	Compound name	%
1.	4.73	Arsenous acid	6.05
2.	5.54	Benzenebutanol	9.45
3.	9.32	2-methyl-3,5-dinitrobenzyl alcohol	7.54
4.	19.41	Triisobutyl (3-phenylpropoxy)silane	16.73
5.	20.63	Purin-2,6-dione	4.88
6.	21.76	Malonic acid	5.81
7.	22.85	5,6-Dihydro-2,4,6-trimethyl-4H-1,3,5-dithiazine	4.86
8.	24.13	N-(3-Allyl-2-oxo-2,3-dihydro-1,3-benzothiazol-6-yl)acetamide	13.04
9.	26.64	Cis-10-heptadecenoic, methyl ester	6.36
10.	26.85	Butyl 9-hexadecenoate	8.74

Figure 2 illustrates GC-MS chromatogram for celery plant.

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

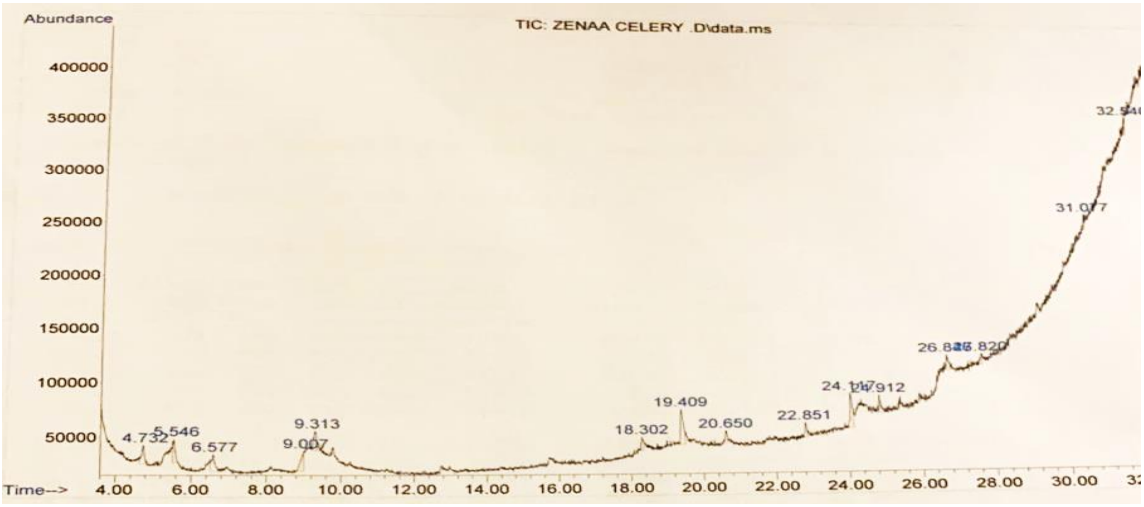

Figure 2: GC-MS chromatograms for celery plant

Table 2: Phytochemical screening for celery plant using GC-MS

Peak No.	RT (min)	Compound	%
1.	4.73	Cyclotrisiloxane	6.96
2.	6.57	Acetamide	7.03
3.	9.00	Propanedioic acid 2-Butanol	9.47
4.	9.31	1,4-Pentanediol Butanal	9.24
5.	19.40	1H-Indole	16.37
6.	24.11	1-Diphenyl(tert-butyl)silyloxy-4-methoxybenzene	13.76
7.	24.91	4-pyridinecarboxamide	4.86
8.	26.84	n-propyl 9-hexadecenoate	4.96

As indicated in table 2, in celery plant, the highest active component was 1H-Indole at a concentration of 16.37% followed closely by 1-Diphenyl (tert-butyl) silyloxy-4-methoxybenzene at a concentration of 13.76%. The least ingredient was 4-pyridinecarboxamide at a concentration of 4.86%. Figure 3 illustrates GC-MS chromatograms for pomegranate plant.

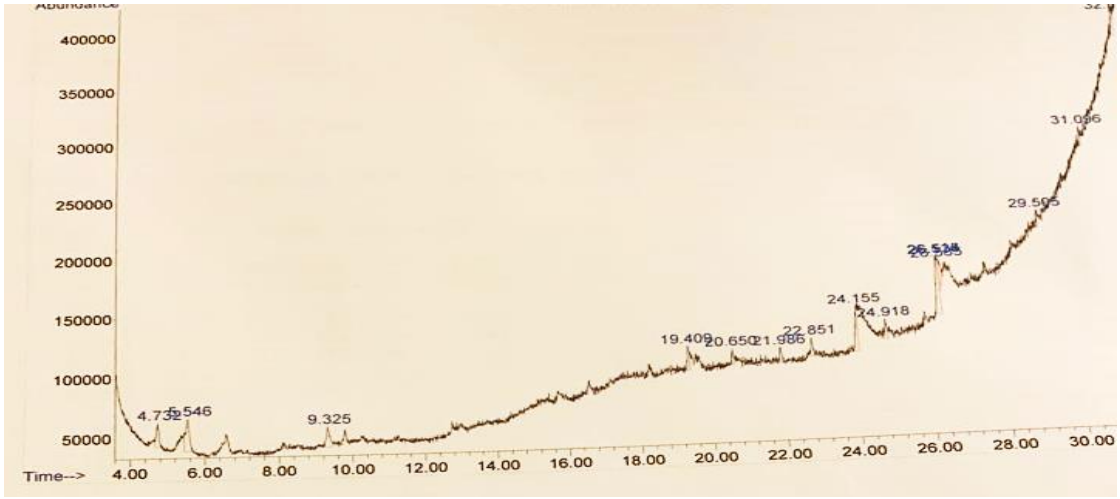

Figure 3: GC-MS chromatograms for pomegranate plant

As shown in table 3, in pomegranate plant, the highest active component was 6-Octadecenoic acid, methyl ester with a concentration of 20.24% followed by 9-Hexadecenoic acid, methyl ester (z)- at 12.36% and benzaldehyde at 10.90%. 1-Decanamine had the lowest concentration at 5.59%.

Table 3: Phytochemical screening for pomegranate plant using GC-MS

Peak No.	RT (min)	Compound name	%
1.	4.73	2,4,6-cycloheptatrien-1-one	5.83
2.	5.54	Benzaldehyde	10.90
3.	9.32	Ethylamine	6.99
4.	19.40	1-Decanamine	5.59
5.	24.15	9-Hexadecenoic acid, methyl ester (z)-	12.36
6.	26.51	6-Octadecenoic acid, methyl ester	20.24
7.	32.58	Fumaric acid	8.37

Identification of phytochemicals in the three plants using HPLC

Table 4: Identification of phytochemicals in celery plant using HPLC

N0.	RT (min)	Compound name	%
1.	34.581	Resverastrol	4.93
2.	37.017	B-Sistine	16.09
3.	40.207	Rutin	5.22

HPLC analysis did not reveal the six standards used in this study for safflower and pomegranate plants however, in celery plant resverastrol, b-sistine and rutin were identified at concentrations of 4.93%, 16.09% and 5.22 % respectively as indicated in table 4. It is worth noting that these three plants elucidated other peaks which could be identified if more known standards are used. This is in contrast to previous reports which have reported quarcetin and rutin content in both safflower and celery plant extracts in their studies [15], [11]. Figure 4, 5 and 6 illustrate HPLC chromatograms from the three plants Celery, Safflower and Pomegranate.

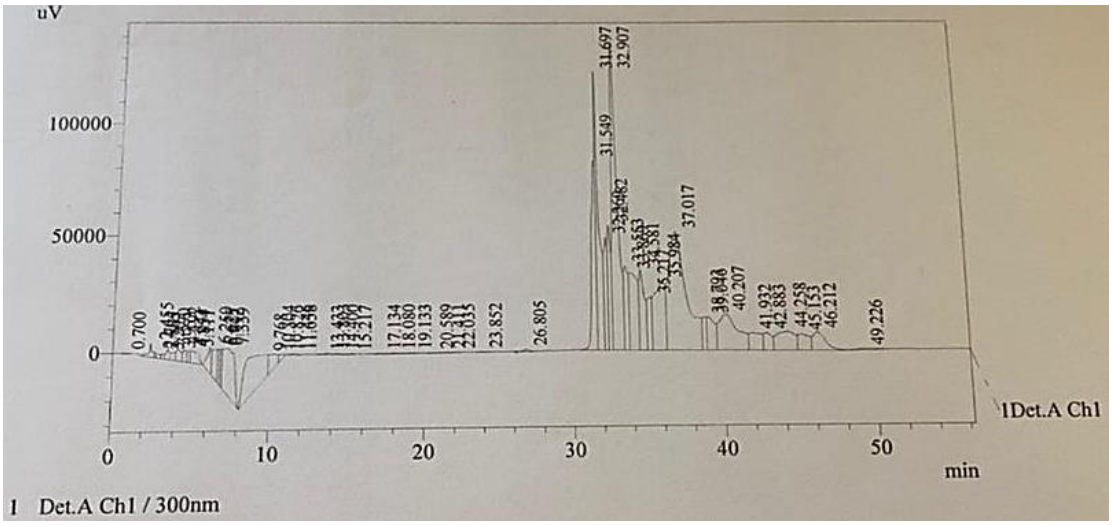

Figure 4: HPLC chromatogram for celery plant

ISSN: 1343-4292
Volume 140, Issue 01, April, 2022

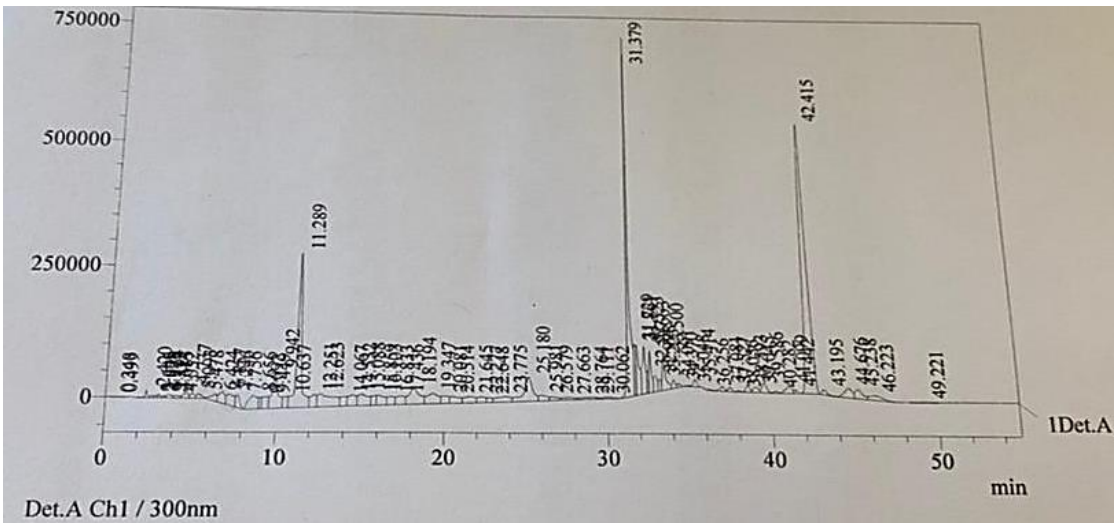

Figure 5: HPLC chromatogram for safflower plant

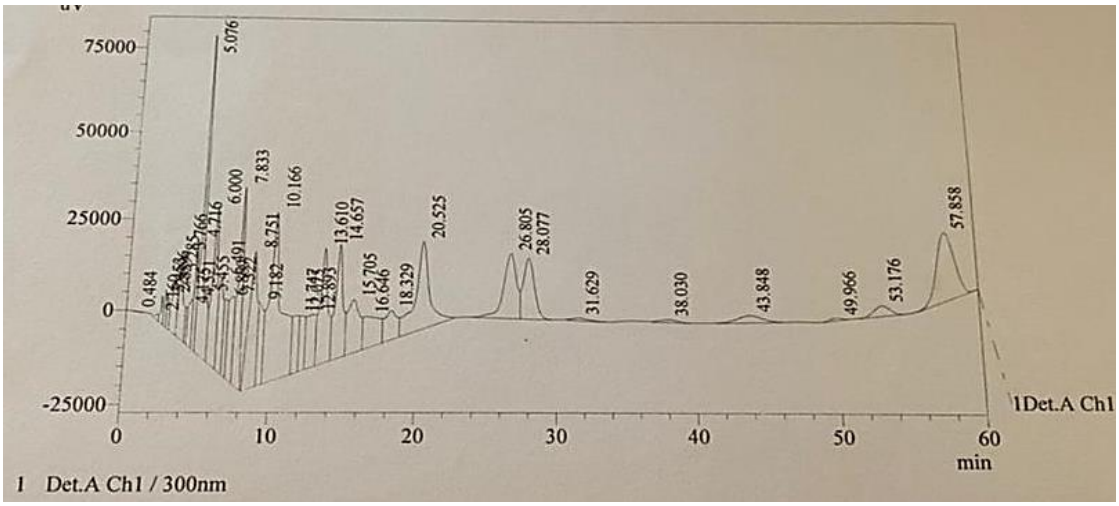

Figure 6: HPLC chromatogram for pomegranate plant

Table 5: Qualitative phytochemical screening for three anti-aging medicinal plants

No.	Test	Celery	Pomegranate	Safflower
1.	Tannins	+	+	+
2.	Carbohydrates	+	+	+
3.	Glycosides	-	+	+
4.	Phenols	-	+	+
5.	Resins	-	-	-
6.	Flavonoids	-	+	+
7.	Saponin	+	+	+
8.	Alkaloids	+	-	-
9.	Protein	-	-	-
10.	Coumarins	-	+	+
11.	Terpenes	-	-	-
12.	Steroids	-	-	-

Qualitative phytochemical screening among the three plants revealed varying results as indicated in table 5. Safflower and pomegranate exhibited similar trend in all the phytochemicals and also there presence was in

abundance relative to celery plant. In celery only four phytochemicals were present tannins, carbohydrates, saponins and alkaloids whereas in pomegranate and safflower resins, alkaloids, proteins, terpenes and steroids were not detected. Previous reports have indicated that pomegranate is rich in polyphenols, mostly ellagic acid and punicalagin [35]. Due to its high level of phenolic compounds, pomegranates has a great antioxidant potential [37] and can lower the risk of acquiring major chronic ailments [7]. [32] has reported that the inherent properties of these compounds serve to prevent various diseases thus reduce the negative attributes associated with aging process.

Table 6: Selected mineral composition of three anti-aging medicinal plants

Plant	Se (ppm)	Zn (ppm)	Ca (ppm)	K (ppm)	Mg (ppm)	Mn (ppm)	Fe (ppm)
Pomegranate	-	0.3674	35.4	1.7	86.4	3.6	70.4
Celery	-	0.3964	153.1	100.1	164.9	27.7	36.4
Safflower	-	1.199	137.1	-	-	-	-

Mineral analysis in the three plants did not reveal selenium ion although zinc was detected but in minute concentrations. Calcium ion was also detected in high concentrations although this is expected because it is a major mineral unlike selenium and zinc which are trace elements or minor minerals. Among the three plants pomegranate exhibited significantly lower concentrations of calcium compared to celery and safflower. Safflower exhibited significantly high zinc content relative to the other plants. Selenium is found in soils naturally but it can also be present as a result of human activities in the environment. Its incorporation into plants results in organoselenium metabolites which based on the molecules nature and the plant species, can be integrated into body proteins [12]. Zinc on the other hand improves the immune system and the body's metabolism function. It also plays a role in wound healing [21]. Calcium is associated with bone density and teethas well as blood clotting and muscle contraction where it is involved in controlling heart rhythms and nerve functions [44].

4. CONCLUSION

GC-MS profiling of the active metabolites among the three plants revealed varying components with different concentrations. On the contrary, HPLC analysis only identified three phytochemicals associated with celery plant while the other compounds were not identified from the six standards used in this study. It is thus recommended that further profiling of these phytochemicals be done using more advanced technology of HNMR. On the other hand, qualitative screening of the different phytochemicals revealed similarity in their occurrence in both pomegranate and safflower plant. Celery plant exhibited the least phytochemicals in occurrence. Lastly, selenium ion was not detected in all the three plants while calcium ion was found to be in high concentrations although pomegranate exhibited a significantly lower concentration.

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