ORIGINAL RESEARCH PAPER
Volume - 13 | Issue - 02 | February - 2024 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

CHEMICAL CHARACTERIZATION OF Allium cepa EXTRACTS

Healthcare

Jegathambigai Rameshwar Naidu*	Associate Professor, International Medical school, Management and science university, Malaysia. *Corresponding Author
Sasidharan Sreenivasan	University Science Malaysia.
Sakina Ruhi	International Medical school, Management and science university, Malaysia.
Danish Khan	Kaloji Narayana Rao University Telangana, India.
Husni Ahmed AL-Goshea	International Medical school, Management and science university, Malaysia.
Aniruddha Bhatterjee	International Medical school, Management and science university, Malaysia.
Sahar Hussain Khan	International Medical school, Management and science university, Malaysia.
Ayesha Syed	International Medical school, Management and science university, Malaysia.
Rajesh Thangarajan	International Medical school, Management and science university, Malaysia.

ABSTRACT

Numerous studies using epidemiological methods have continuously connected a high intake of plant-based diets with a lower risk of cancer. The present study was conducted to perform the chemical and phytochemical characterization of *Allium cepa* extracts. Qualitative phytochemical analysis, HPLC analysis and GC-TOFMS analysis were used for the study. The results from the phytochemical analysis indicated the presence of saponin, anthroquinone, flavonoid and alkaloid in *Allium cepa* extracts. In addition, GC-spectrum of the *Allium cepa* extracts indicated the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol, glycosides and caryophyllene. The major phytochemicals identified in the plant investigated may be recommended as good candidates for cytotoxicity and antiangiogenic potential.

KEYWORDS

Allium cepa, Phytochemical, chemical characterization, flavonoids, quercetin, alkaloid.

INTRODUCTION

Analysis of the bioactive components of plants is essential for the creation of botanical pharmaceuticals, including traditional herbal remedies, as the therapeutic properties of plants rely on the bioactive phytochemical constituents that have specific physiological actions in the human body. Alkaloids, flavonoids, tannins, saponins, and cardiac glycosides have all been found in therapeutic plants using qualitative and quantitative phytochemical screening (1). The number of novel bioactive chemicals is rising because of developments in spectroscopy, chromatography, and other separation methods. *Allium cepa* is an evergreen bulb growing to 0.6 m (2 feet). It is the edible plants of various *Allium* species, all of which are "onion-like", having hollow green leaves and lacking a fully developed root bulb. The leaf blades are tubular, slightly flattened on the adaxial side, and although hollow is closed at the tip. Leaves are up to 40 cm in height and 20 mm in diameter. The importance of biological and pharmacological activities, such as antifungal, antibacterial, antitumor, anti-inflammatory, antithrombotic and hypocholesterolemic properties of certain steroid saponins and sapogenins, such as b-chlorogenin, has been recently demonstrated (2).

In plant extracts, gas chromatography (GC) or gas chromatography-mass spectroscopy (GC-MS) are nearly the only methods utilized for qualitative investigation of volatiles and complicated combinations of bioactive chemicals (3). Extensive two-dimensional gas chromatography (GC) has been successfully utilized in the industrial examination of plant materials to enhance component separation and identification, and it has also been applied extensively in the research of essential oils (4). The three main mass spectrometry techniques used for metabolite analysis are now gas chromatography-mass spectrometry, gas chromatography–time-of-flight–mass spectrometry (GC-TOF-MS), and liquid chromatography–mass spectrometry (LC-MS) (5). The comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection (GC×GC-TOFMS) is the most advanced analytical technique of high sensitivity and selectivity. It is also a technique which provides a substantial enhancement of peak capacity and signal intensity over conventional GC analysis (6, 7).

For the qualitative and quantitative analysis of phenolic compounds, luteolin, lithospermic acid, and other compounds from thyme, wild thyme, and sweet marjoram, HPLC and HPTLC techniques were frequently employed (8). Using selective determination of positive mode electrospray ionization and HPLC-UV-MS/MS, quercetin glucoronides have been found in human plasma samples (9). According to a recent study, luteolin, epicatechin, rutin, and catechin were all simultaneously isolated using HPLC analysis (10).

Flavonoids, alkaloids, phenolics, essential oils, tannins, and saponins are examples of polyphenols, which constitute the primary class of phytochemicals with possible health advantages (11). In vegetables, flavonoids are found in a large diversity. Vegetables have a significant subclass of flavonol-type flavonoids, which includes myrecitin, kaempferol, and quercetin as well as their glycosides (12). It is crucial to identify the polyphenols that are already present in fruits, vegetables, and culinary herbs that are likely to provide health advantages to determine the impacts of polyphenol consumption and health benefits. In view of this, it would be useful to identify the commonly occurring beneficial flavonoids such as quercetin, rutin, kaempferol and caffeic acid in *Allium cepa* extracts. In addition, chemical profiling of the extracts would help us to identify lead structures with pharmacological potential. On this direction the present study was done to perform the phytochemical analysis and chemical profiling of the crude extracts of *Allium cepa.*

MATERIALS AND METHODS

Plant Sample Collection And Preparation

Allium cepa were collected from the small farms around the Bedong

and Semeling regions of Sungai Petani. The plants were identified by Botanist, and voucher specimens were submitted to the herbarium unit. The aerial parts of the procured plant samples were washed thoroughly under running tap water and dried at room temperature (30°C) for a week. Finally the dried plant material were grounded to a fine powder using an electric blender and transferred to clean dry glass containers and stored in dark until further use.

Extraction Of Plant Material

200 g of the finely powdered plant material of the selected plants were macerated and soaked in 80% methanol for 4 days. The extracts were then clarified by filtration through filter paper (Whatman No.1) and is then concentrated *in vacuo* using a rotary evaporator at 40C to give the respective crude extracts. The crude extracts were then weighed and transferred into vial wrapped with aluminum foil and stored at 4C to prevent loss of material until further use (13).

Qualitative Phytochemical Analysis

Qualitative phytochemical tests were done to identify the presence of sugar, tannins, flavonoids, anthocyanins and alkaloids in the crude extracts. Several chemical reagents were used in the detection according to the previously published methods (14, 15).

Preparation Of The Plant Sample

About 100 mg/ml of crude extracts of the selected plants were prepared by diluting in methanol for the Benedict's test, Borntrager's test, flavonoid test, ferric chloride test and alkaloid test. For the saponin test the dried crude extracts were used

Benedict's Test

To 2.5 ml of the Benedict's reagent, added 2 ml of the diluted crude extracts of the selected plants respectively and the mixture is vortexed and heated in a boiling water bath for 10 min. Formation of a brick red precipitate was recorded as a positive result (+) for the presence of reducing sugar.

Frothing Test

0.1 g of the crude extracts of the selected plants were diluted with 1 ml distilled water respectively in test tubes and shaken vigorously for 1 min and let to stand for 10 min to observe the persistent froth formation in the tube, frothing was recorded as a positive result (+) for the presence of saponins.

Borntrager's Test

To 2 ml of the diluted plant extracts of the selected plants added 1 ml of the diluted ammonia (10%) and shaken well for few seconds to observe the color change. The formation of bright pink color was recorded as a positive result (+) for the presence of anthroquinones.

Flavonoid Test

To 2 ml of the diluted plant extracts of the selected plants added 2 ml of diluted sodium hydroxide and a few drops of concentrated HCL. The formation of a pink or red color solution was recorded as a positive result (+) for the presence of flavonoids.

Ferric Chloride Test

To 2 ml of the diluted plant extracts of the selected plants added 1ml of 15% ferric chloride solution respectively, in test tubes, shaken well and the color change was noted. Formation of a blue or green blackish precipitate was recorded as a positive result (+) for the presence of tannins.

Alkaloid Test

To 2 ml of the diluted plant extracts of the selected plants added 10 ml of ammoniacal chloroform, and few drops of 10% sulphuric acid and tested with Meyer's reagent. The formation of a white precipitate indicated the presence of alkaloid and recorded as a positive result (+).

GC-TOFMS (Gas-chromatography-time-of-flight-mass-spectrometer) analysis

GC-TOFMS is the most advanced analytical technique of high sensitivity and selectivity. It also provides a substantial enhancement of peak capacity and signal intensity over conventional GC analysis (6). For GC-TOF-MS analysis, 0.1 g of crude extract was mixed with 1 ml of distilled water and 4 ml of solvent mixture (ethyl acetate:hexane:methylene chloride). The mixture was agitated for 2 min at 3000 rpm. The supernatant was filtered and injected into the GC column. The chemical profiling of the crude extracts of the plants was done by using a time-of-flight mass spectrometer (TOFMS) (Pegasus®) for GC/MS analysis. The m/z of each ion is determined by its time of flight and equation (t = Slope*(m/z)1/2 + Offset). Slope and offset values were determined using a mass calibration standard which was done automatically by the mass calibration routine of the Pegasus® Chroma TOF TM software (15).

GC-TOF MS Instrumentation Parameters

The instrumentation parameters for GC-TOF MS are listed in in Table 1.

Table 1 Instrumentation Parameters For GC -TOFMS

Model	Leco Pegasus III
Detector	Leco Pegasus III Time-of-flight Mass Spectrometer
Software	ChromaTOFSoftware
Transfer Line	260ºC
Ion Source	250ºC
Acq. Rate	10 spectra/sec (35 to 550 amu)
GC	Hewlett Packard 6890
Column	DB-5 20 x 0.18 mm ID, 0.18 µm phase film
Oven	50 (kept 2 min) to 250ºC (kept 10 min),with a rate of 8ºC/min
Injector	250ºC
Carrier gas	Helium, 1.2 ml/min, constant flow
Sample	1.0 µL, split injection

HPLC determination of polyphenols (quercetin, rutin, kaempferol, caffeic acid)

HPLC Instrumentation And Operating Conditions

HPLC Instrumentation

HPLC-determination was carried out on a Waters 2695 separation module which consists of an integrated quaternary solvent delivery system and sample management platform. Automated sample injection systems and multiport injection valves have good reproducibility so that a series of injections can be made with a variation in sample volume less than 1 %.

Sample Preparation

Crude Extracts

0.1 g of the solvent free crude extracts were re-dissolved in 5 ml of 100% methanol. The extracts were filtered through 0.20 m micro-filter. 20 l was injected into the HPLC.

Standards

1 mg of the standards (quercetin, rutin, kaempferol, caffeic acid) were diluted serially (1250, 2500, 5000, 7500 µg/ml) in HPLC grade acetonitrile and filtered through 0.2 m micro-filter. 20 l was injected into the HPLC.

Operating Conditions

The column used was a reverse-phase C18 Novapak column (4.6 x 250 mm I.D; 5m). A two solvent gradient system was used. The optimized mobile phase was (A) water: formic acid (99:1) and (B) 49% water, 50% methanol, 1% formic acid. The sample injection volume was 20 l. Gradient elution was performed at a flow rate of 1 ml / min for 30 min. The detector monitored the sample at 254.9 nm for quercetin, 256.1 nm for rutin, 230.1 for kaempferol and caffeic acid. The identification of the compounds in the samples were achieved by comparison of both retention time (tR) values and absorption spectra obtained for each eluted peak of the samples with those obtained for external standards quercetin, rutin, kaempferol and caffeic acid purchased from Sigma chemicals (16).

Quantification Of Polyphenols (quercetin, Rutin, Kaempferol, Caffeic Acid)

Quantification of polyphenols was performed based on the external standards with a mixture of standards of known concentration that were analyzed in duplicates before and after the batch of the samples and their peak area was recorded. The peak area was used to calculate the concentration of the compounds in the analyzed samples. The amount of the polyphenols in the plant samples was determined by the peak area, concentration of the external standards used by using the below calculation.

Concentration of sample = Concentration of external standard / Peak area of external standard x Peak area of unknown.

RESULTS

Extraction Of The Plant Material

The percentage recovery of the crude extracts obtained were found to be 6.96% respectively for Allium cepa extracts. (Table 2).

Table 2 Percentage Recovery Of Crude Extracts

Name of the plant	Dry weight of the Plant samples (g)	Weight of crude extracts (g)	Percentage recovery (%)
Allium cepa	200	13.92	6.96

200 g of the finely powdered plant material of the selected plants were macerated and soaked in 80% methanol for 4 days. The extracts were then clarified by filtration through filter paper (Whatman No.1) and is then concentrated *in vacuo* using a rotary evaporator at 40C to give the respective crude extracts. The extracts were weighed, and the percentage recovery was calculated for 200 g of dried plant material.

Phytochemical Analysis

Results from the phytochemical analysis indicated the presence of saponin, anthroquinone, flavonoid and alkaloid in *Allium cepa* extracts **(Table 3)**

Table 3 Phytochemical Analysis Of The Plant Extracts

Plant	Benedict's test	Frothing test	Borntrager's Test	Flavonoid test	Ferric chloride test	Alkaloid test
Allium cepa	+	+	+	+	-	+

(+ indicates the presence and - indicates the absence of the organic compounds tested by qualitative color reaction. (Benedict's test- reducing sugar, frothing test- saponins, Borntrager's test-anthro-quinones, flavonoid test-flavonoids, ferric chloride test-tannins, alkaloid test-alkaloids)

GC-TOFMS (Gas-chromatography-time-of-flight-mass-spectrometer) Analysis

GC Spectrum Of *Allium Cepa*

GC-spectrum of the *Allium cepa* extracts indicated the presence of fatty acid methyl esters (hexa decane, hepta decane, octa decane) terpenoids, terpenoid alcohol, glycosides and caryophyllene. Table-3.8 represents the compounds identified, chemical formula, peak area (%) and retention time (s) of the crude extracts of Allium *cepa.* Figure 3.5 represents the GC spectrum of Allium *cepa* which indicated the presence of 36 compounds as shown in Table 4

Table 4 Chemical Composition Of *Allium Cepa*

NO	Name of the compound	Formula	% Area	R.T (s)
1.	(R)-(-)-2-Amino-1-proponal	C_3H_9O	1.88	194.942
2.	1,6,3,4-Dianhydro-2-deoxy-ă-d-lyxo-hexopyranose	$C_3H_8O_3$	0.35	1658.54
3.	10-methyl-8-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	0.01	1696.97
4.	1-Hexacosene	$C_{26}H_{22}$	1.45	1583.62
5.	2,5-Dimethoxy-4-(methylsulfonyl) amphetamine	$C_{12}H_{19}NO4S$	2.95	479.99
6.	2-Piperidinone, N-(4-bromo-n-butyl)-	$C_9H_{16}BrNO$	0.11	1590.21
7.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	18.52	1335.53
8.	4-Methyldecosane	$C_{23}H_{48}$	2.45	1574.29
9.	5-Nonyl-1-H-(1,2,4)-o-triazol-3-ylamine	$C_{11}H_{22}N_4$	0.11	982.953
10.	6-Azacholest-4-ene-7-one, 6-benzyl-3ă-hydroxy-	$C_{33}H_{49}NO_2$	0.66	1445.89
11.	7,8-Epoxylanostan-11-ol,3-acetoxy-	$C_{32}H_{54}O_4$	1.31	1604.93
12.	7-Hexadecenal	$C_{16}H_{30}O$	0.07	1503.43
13.	9, 12, 15 Octadecatrienoic acid, methyl ester (Z, Z,Z)-	$C_{19}H_{32}O$	1.33	1327.34
14.	ă-Caryophyllene	$C_{15}H_{24}$	0.53	815.188
15.	Actinobolin	$NH_{20}N_2O_6$	0.15	1157.11
16.	Amphetamine-3-methyl	$C_{10}H_{15}N$	3.58	205.931
17.	Bactobolin	$C_{14}H_{20}C_{12}N_{26}$	1.90	1677.99
18.	Benzeneethanamine, 3-fluro-ă, 5-dihydroxy-N-methyl-	$C_9H_{12}FNO_2$	3.15	199.737
19.	Bufa-20, 22-dienolide,14,15-epoxy-3, 11-dihydroxy-,(3ă, 5ă-, 11ă-, 15ă)-	$C_{24}H_{32}O_6$	18.52	400
20.	Caryophyllene	$C_{15}H_{24}$	9.28	785.018
21.	Caryophyllene oxide	$C_{20}H_6N_4$	0.24	931.536
22.	Cyclohexane, 4-ethenyl-4-methy-3-(1-methylethynyl)-1- (1-methylethyl)- (3R-trans)-	$C_{15}H_{24}$	0.01	703.233
24.	Cyclohexane, 4-metheylene-1-(1-methylethyl)-	$C_{10}H_{16}$	0.66	310.56
25.	Decane, 3,8-dimethyl-	$C_{12}H_{26}$	3.86	1569.23
26.	Dodecane,1-fluro	$C_{12}H_{25}F$	0.29	1690.78
27.	Dodecane, 2, 6,10-trimethyl-	$C_{15}H_{32}$	0.01	1524.74
28.	Eicosane	$C_{20}H_{42}$	0.19	1480.12
29.	Epinephrine	$C_9H_{13}NO_3$	2.70	890.846
30.	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	6.04	1219.45
31.	Glycyl di alanine	$C_5H_{10}N_2O_3$	2.06	461.409
32.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	1.16	1203.67
33.	Naphthalene, 1,2,3,4,4â,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2â4aâ,8aâ)}-	$C_{15}H_{24}$	2.06	845.491
34.	Naphthalene,1,2,3,4,4â,5,6,8a-octahydro-7-dimethyl-4-methylene-1-(1-methylethenyl)-, (1â4aâ,8aâ)}-	$C_{15}H_{24}$	1.00	741.662
35.	Phenylethyamine, p, â-dimethyl-	$C_{10}H_{15}N$	3.67	771.432
36.	Tungsten, dicarbonyl-(Q-4-pinocarvone)[1,2-bis(dimethylphosphino)ethane	$C_{18}H_{30}O_3P_2W$	0.01	1215.19

Represents the compounds identified, chemical formula, peak area (%) and retention time (s)

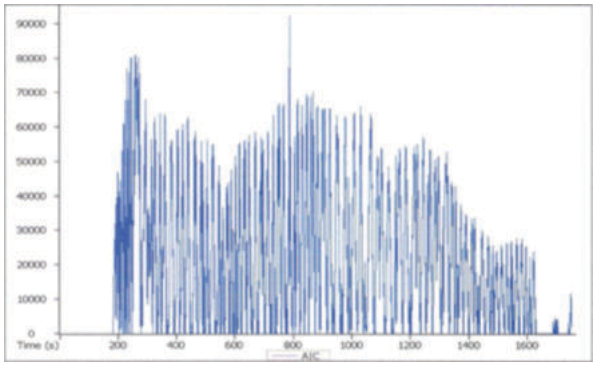

Figure 1 GC spectrum of the crude extracts of *Allium cepa*

HPLC determination of polyphenols (quercetin, rutin, kaempferol, caffeic acid)

The HPLC profile indicated the presence of the flavonoids, quercetin and rutin, in *Allium cepa* extracts. Caffeic acid was found to be present in *Allium cepa.* The retention time of quercetin was 21.769 for *Allium cepa* when compared to the retention time of standard quercetin (20.944 min). Meanwhile, the retention time of rutin was found to be 18.361 for *Allium cepa* when compared to the retention time of standard rutin (17.899 min). (Table 5 and Fig 2).

Table 5 HPLC Chromatogram, Retention Time Of Poly Phenols In AC

Plant	Phenols	Retention Time (min)
Allium cepa	Quercetin	21.769
	Rutin	18.361
	Kaempferol	nd
	Caffeic acid	14.478

HPLC chromatogram of AC-*Allium cepa* compared to standards. Detected under different wavelengths: 254.9 nm for quercetin, 256.1 nm for rutin, 230.1 nm for kaempferol and caffeic acid.

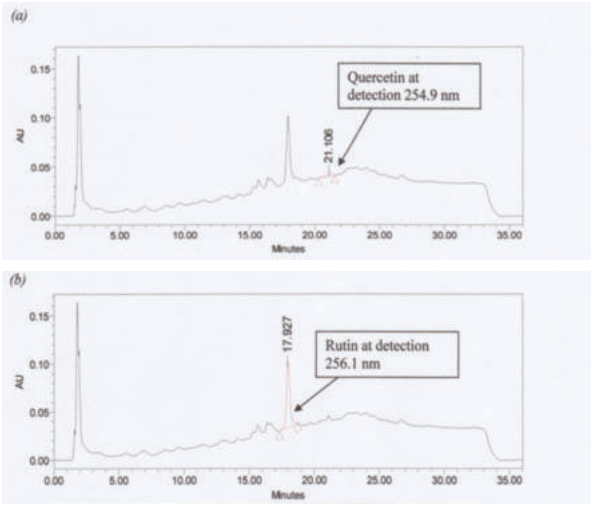

Figure 2 HPLC Chromatogram Of *Allium Cepa*

Different detection wavelength. a: 254.9 nm for quercetin with a retention time of 21.106 min, b: 256.1 nm for rutin with a retention time of 17.927 min.

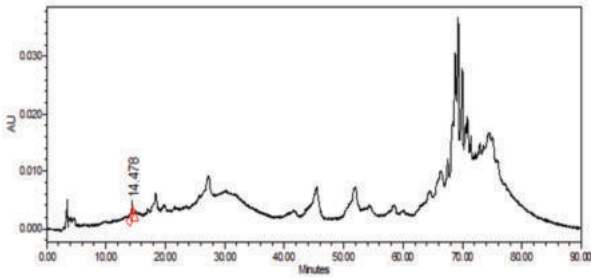

Figure 2 Continued

c: 230.1 nm for caffeic acid with a retention time of 14.478 min.

Quantification of polyphenols (quercetin, rutin, kaempferol, caffeic acid)

HPLC quantification of the polyphenols (quercetin, rutin, kaempferol, caffeic acid) was done based on the method described earlier in Section 3.2.6. The results are shown in Table 6.

The concentration of quercetin was found to be 107.82, µg/g in Allium cepa extracts The concentration of rutin was found to be 1663.73 µg/g in the crude extracts of *Allium cepa* respectively. The concentration of caffeic acid was found to be 4.16 µg/g in the crude extracts of *Allium cepa* Kaempferol was not detected in *Allium cepa* extracts.

Table 6 Quantification Of Polyphenols In AC

Plant	Flavonoids	Concentration of phenols (µg/g)
Allium cepa	Quercetin	107.82
	Rutin	1663.73
	Kaempferol	nd
	Caffeic acid	4.16

Nd: not detected

Quantification of polyphenols was performed based on the external standards with a mixture of standards of known concentration that were analyzed in duplicates before and after the batch of the samples. The peak area was used to calculate the concentration of the compounds in the analyzed samples.

DISCUSSION

Preliminary phytochemical and chemical characterization of the plant extracts were done to identify selected bioactive compounds. Initially the powdered plant material was macerated with 80% methanol to obtain the crude extracts. Extraction of the plant material by standardized protocol is an essential step to isolate the organic constituents from the plant. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. Alcohol is favored for the extraction of plant materials as it facilitates the complete extraction of the various organic compounds with different polarities and at lower temperatures and boiling point compared to aqueous extraction (14)

Organic solvents like methanol and ethanol are efficient to extract volatile and saturated organic compounds from plant materials due to less contamination problems during storage and easier evaporation compared to water extracts. Studies have reported a higher percentage yield of crude extracts by methanol extraction when compared to non-polar solvents (17; 18) Methanol and ethanol have been extensively used to extract antioxidant compounds from various plants and plant-based foods (fruits, vegetables etc.) such as plum, strawberry, pomegranate, broccoli, rosemary, sage, sumac, rice bran, wheat grain and bran, mango seed kernel, citrus peel, and many other fruit peels (19).

Preliminary qualitative phytochemical tests were done to identify the presence of sugar, tannins, flavonoids, anthocyanins and alkaloids in *Allium cepa* extracts. Several chemical reagents were used for this following the previously published methods (13,14). Results from the phytochemical analysis indicated the presence of saponin, anthroquinone, flavonoid and alkaloid. Certain steroid saponins and sapogenins, such as b-chlorogenin, has been recently demonstrated in *Allium cepa* (20).

The chemical profiling of the crude extracts of the plants was done by using a time-of-flight mass spectrometer (TOFMS) (Pegasus®) for GC/MS analysis. The m/z of each ion is determined by its time of flight and equation (t = Slope*(m/z)1/2 + Offset). Slope and offset values was determined using a mass calibration standard which is done automatically by the mass calibration routine of the Pegasus®Chroma TOFTM software (15). The rapid advances in spectroscopic and chromatographic techniques have totally changed the picture of chemical study of plants and essential oils. Many techniques have been used for studying the chemical profiles of plants and essential oils; e.g. IR-spectroscopy, UV-spectroscopy, NMR spectroscopy and gas chromatography (21,22). Gas chromatography has been proved to be an efficient method for the characterization of plant constituents and essential oils 23 (Anwar *et al*., 2009). The combination of gas chromatography and mass spectrometry (GC-MS) allows rapid and reliable identification of essential oils components from plants (24, 23). The most advance of these methods, comprehensive GC×GC-TOFMS, was successfully used for separation of many groups of natural compounds or xenobiotics (25, 26). Time-of-flight mass spectrometry (TOFMS) is probably the simplest method of mass spectrometric measurement by the physical principle. The key features of TOFMS are extreme sensitivity (all ions are detected), practically unlimited mass range and as well as high-speed analysis recent TOFMS instruments are able to measure hundreds full spectra per second) which makes it one of the most desirable methods of mass analysis (27).

The GC spectrum of *Allium cep*a extracts indicated the presence of triterpeniod and triterpene alcohols, steroid derivatives (7,8-epoxylanostan-11-ol) fatty acid methyl ester (9, 12, 15 octadecatrienoic acid, methyl ester (Z, Z,Z)-), sulphur compounds (2,5-dimethoxy-4-(methylsulfonyl)amphetamine), aromatic alkanes, amines and alkenes (amphetamine-3-methyl, benzeneethanamine, caryophyllene, decane, 3,8-dimethyl, epinephrine, phenylethyamine, p-â-dimethyl- tungsten, dicarbonyl-(Q-4-pinocarvone) [1,2-bis(dimethylphosphino) ethane) and amino acids (glycyl di alanine). Fatty acid methyl esters and amino acids have been reported in the present study.

The biological effects of intact garlic and onion, such as lectins (the

most abundant proteins in garlic and onion), prostaglandins, fructan, pectin, adenosine, vitamins B1, B2, B6, C and E, biotin, nicotinic acid, fatty acids, glycolipids, phospholipids and essential amino acids, have been studied for over several decades. 7,8-epoxylanostan-11-ol, a steroid derivative was found to be present in *Allium cepa* extracts. The importance of biological and pharmacological activities, such as antifungal, antibacterial, antitumor, anti-inflammatory, antithrombotic and hypocholesterolemic properties of certain steroid saponins and sapogenins, such as b-chlorogenin, has been recently demonstrated (20). The present study reported the presence of sulphur compounds (2,5-dimethoxy-4-(methylsulfonyl) amphetamine), in accordance with these high levels of the reduced organosulfur compounds alkenyl cysteine sulfoxides (ACSOs), which confer characteristic flavors and human health benefits (28).

Diets rich in selected natural antioxidants such as polyphenols, flavonoids, vitamin C and vitamin E are related to reduced risk of incidence of cardiovascular, other chronic diseases and certain types of cancer has led to the revival of interest in plants-based foods (29) Polyphenols especially flavonoids that are rich in fruits, soybeans, vegetables, roots and leaves has a role in prevention of heart and cardiac disease (30). Fresh green tea contains large amount of catechin, polyphenols, while resveratrol and quercetin are rich in grapes, red wine and other food products (31). Various kinds of Thai vegetables including bitter guard, turmeric, ginger, garlic, basil leaves, citrus leaves and lemon grass have shown to possess antimutagenic chemicals which induced detoxifying enzymes such as glutathione-S-transfrease inhibiting carcinogenesis (32).

HPLC analysis was done to confirm the presence polyphenols, including flavonoids such as quercetin, rutin, kaempferol and caffeic acid. For the HPLC determination, a rapid and simple reverse-phase HPLC method was developed by using a C18 Novapak column (4.6 x 250 mm I.D; 5m) with a PDA detector. Gradient elution was performed at a flow rate of 1 ml / minute for 30 minutes. The detector monitored the sample at 254.9 nm for quercetin, 256.1 nm for rutin and 230.1nm for kaempferol and caffeic acid. A recent study has reported the identification of quercetin glycosides in human plasma by reverse-phase HPLC-UV-MS method at 254 nm using a PDA detection system (33). The identification of the compounds in the samples were achieved by comparison of both retention time (tR) values and absorption spectra obtained for each eluted peak of the samples with those obtained for external standards quercetin, rutin, kaempferol and caffeic acid purchased from Sigma chemicals (16) A reverse-phase HPLC has been used in a number of occasions for the analysis of flavonoids in plants, it a used to distinguish species based on the quantitative variation of flavonoids among them. It has been applied especially for the identification of flavonoid derivatives. Flavonoids were quantified using 254 nm using peak area by comparison to a calibration curve derived from the quercetin. Seven flavonoid compounds including quercetin and its glycosides have been isolated from flowers of A.indicum (34) HPLC coupled with diode-array detection was used to identify and quantify the phenolic compounds such as rosmarinic acid, quercetin, and kaempferol in selected culinary herbs and medicinal herbs. To accomplish the selectivity and specificity detection required to identify quercetin and its metabolites at trace levels in complex biological matrices, preliminary experiments had proved that HPLC-MS are superior when compared to UV or electrochemical detection (35).

The HPLC profile indicated the presence of quercetin, rutin and caffeic acid in Allium cepa. The concentration of quercetin was found to be 107.82,µg/g in the crude extracts of Allium cepa respectively. The concentration of rutin was found to be 1663.73 µg/g in the crude extracts of Allium cepa respectively. The concentration of caffeic acid was found to be 4.16 µg/g in the crude extracts of *Allium cepa* respectively.

Report from a previous study indicated the presence of quercetin in *Allium cepa* (1497.5 mg/kg), *Mentha arvensis* (48.5 mg/kg) and *Centella aiatica*: kaempferol (178 mg/kg) in *Cymbopogan citratus and Centella asiatica.* Querectin was not reported in the previous study in *Cymbopogan citratus* extracts (36). The highest total flavonoid content as found to be in *Ocimum basilicum* followed by *Mentha spicata*, *Cymbopogan citratus* and *Allium cepa.* Studies have reported highest total flavonoid content in onion leaves and trace quantities in *Mentha spp.* Allium vegetables (onion leaves, chinese chive leaves and garlic) contained quite high flavonoid content The flavonoid content reported in the present study was found to be higher than previously reported studies (37).

Flavonols in the edible portion of the *Allium* vegetables (leeks, shallots, green onions, garlic and onions) range from less than 0.03 to 1 g/kg, white onions contained no detectable flavonols when compared to yellow and red onion which contained 60-1000 mg/kg (38). A report on the locally consumed vegetables including pegaga, semambu, papaya shoot, cekur manis, belimbi leaves, cashew shoot, kesom leaves indicated high flavonoid content. Among flavonoids, one of the major subgroups ubiquitously occurring in vegetables is flavanol-type flavonoids including kaempferol, quercetin and myrecitin and their glycosides (39). Quercetin glucosides and rutin are present in onion and common vegetables. In vegetables quercetin glycosides predominant, but glucosides of kaempferol, luteolin and apigenin are also present. The difference in the reported flavonoid content may be attributed to the difference on the method employed, lack of precision and accuracy, parts of the plants used, cultivars or varieties used. The concentration of secondary metabolites in plants are dependent on certain factors such as growing condition, size, degree of ripeness and variety (40). Phenolic compounds are usually affected by temperature changes and acidic conditions during the extraction process. Drying below 50˚C yields highest phenolic compounds, increasing temperature above 60˚C lowers the phenolic content.

Quercetin is a potent anticancer agent and antioxidant that can contribute to the prevention of atherosclerosis (41). Quercetin is a chemopreventive and chemotherapeutic agent with anti-inflammatory potential and anti-tumor potential that can reduce mutagenicity of cooked foods and enhance the action of anticancer agents and inhibit the growth of tumorigenic cells 50 (Leighton *et al*., 1992). Dietary quercetin glycosides as found to induce the anticarcinogenic phase II marker enzyme quinone reductase in Hepalclc7 cells (42). Kaempferol has antioxidant, antitumor, anti-inflammatory and anti-ulcer activity (43). Caffeic acid, both esterified and free form is generally the most abundant phenolic acid and represents 75% of the total hydroxy cinnamic acid content of fruits. Certain types of polyphenols such as quercetin are found in all plant products (fruits, vegetables, cereals, leguminous plants, tea, wine etc.

Over the past decade, researchers and food manufacturers have become increasingly interested in polyphenols due to their abundance in our diet and their probable role in the prevention of various diseases associated with oxidative stress, such as cancer and cardiovascular and neurodegenerative disease. Studies have suggested that dietary flavonoids are helpful in the prevention of atherosclerosis and cardiovascular disease. Antioxidant activity should be noted as an underlying mechanism of their health impact in the vascular system, as atherosclerosis is closely related to oxidative events such as oxidized LDL accumulation in the macrophages (39). Epidemiological data have clearly indicated the negative relationship of consumption of plant-based foods, such as fruits, vegetables and legumes and cardiovascular diseases (44). A wide range of phytochemicals including (flavanone, flavanol, flavone, isoflavone, phenolic acid and phenylpropanone family have been shown to have protective effects on oxidative damage on cell membranes. High phenolic content especially flavonoids are major contributor for the antioxidant activity (45). The cardiovascular protective effect of these dietary antioxidants may be attributed directly to limiting secondary intracellular ROS generation induced by oxidative stress as proven by some dietary antioxidants including quercetin and rutin (46).

Quercetin glucuronides have been hydrolyzed by cell-free extracts of human neutrophils, liver and small intestine. The enzyme β-glucoronidase favors glucoronidation of flavonoids rather than deglucoronidation at physiologic pH. The activity of this enzyme is increased in physiopathologic states such as inflammation, cancer, AIDS (47). Since polyphenols are metabolized by the same pathway as xenobiotics, they may have indirect health effects. Flavonoids could act as cytochrome P450 inhibitors and enhance drug bioavailability, polyphenols could affect the bioavailability of many carcinogens, other toxic chemicals and therapeutic drugs by affecting the various enzymes involved in them on metabolism (48).

CONCLUSIONS

In conclusion, the results from the phytochemical analysis indicated the presence of saponin, anthroquinone, flavonoid, alkaloid in *Allium cepa* extracts.

Since the major phytochemicals and chemicals identified in the tested plants are found to be chemopreventive and chemotherapeutic agents with antioxidant, anti-inflammatory potential and anti-tumor potential, the plants investigated may be recommended as good candidates for cytotoxicity and antiangiogenic potential. Further studies targeting the antioxidant, cytotoxic and antiangiogenic activity of *Allium* extracts would be helpful to identify lead structures with chemopreventive and pharmacological potential.

Acknowledgements
INFORMM, University Science Malaysia, Malaysia

REFERENCES

1. Gurinder J Kaur and Daljit S Arora. (2009). Antibacterial and phytochemical screening of Anethum *graveolans, Foeniculum vulgare* and *Trachyspermum ammi. BMC Complementary and Alternative Medicine,* 9: 30.
2. Lanzotti et al. Lanzotti, V. (2006). Analysis of onion and garlic. Journal of Chromatography A, 1112.
3. Zhao, Z.L., Dorje, G., Wang, Z.T. (2010). Identification of medicinal plants used as Tibetan medicine Jie-Ji. Journal of Ethnopharmacology 132, 122–126.
4. Shellie, R., L. Mondello, P. Marriott and G. Dugo. (2002). Characterisation of lavender essential oils by using gas chromatography-mass spectrometery with correlation of linear retention indices and comparision with comprehensive two-dimentional gas chromatography. Journal of Chromatography A. 970: 225-234.
5. Alisdair et al., 2004). Alisdair, R. Fernie., Richard, N. Trethewey., Arno, J. Krotzky and Willmitzer. (2004). Metabolite profiling: from diagnostics to systems biology Nature Reviews. Molecular Cell Biology Volume 5 September 1-7.
6. Michal et al., 2012; Michal, Hoskovec., Blanka, Kalinová and Miloš Knížek. (2012). Chiral and Nonchiral GC×GC/TOFMS Analysis of Natural Compounds: The Case of Possible Aggregation Pheromones of Chinese Bark Beetles Ips shangrila and I p s nitidus.
7. Mondello, L.; Tranchida, P.Q.; Dugo, P. & Dugo, G. (2008). Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. Mass Spectrometry Reviews, Vol. 27, No. 2, pp. 101–124.
8. Izabela, Fecka., Sebastian, Turek. (2008). Determination of polyphenolic compounds in commercial herbal drugs and spices from Lamiaceae: thyme, wild thyme and sweet marjoram by chromatographic techniques, Food Chemistry 108. 1039–1053.
9. Jőrg, Wittig., Markus, Herderich., Eva, Ulrike, Graefe., Markus, Veit. (2001). Identification of quercetin glucuronides in human plasma by HPLC-tandem mass spectrometry. *Journal of chromatography B, 753*: 237-243.
10. Bimakr Mandana., Russly, Abdul. Rahman., Farah, Saleena., Taip, Ali Ganjiloo., Liza Md, Salleh., Jinap, Selamat., Azizh, Hamid., I. S.M. Zaidul. (2011). Comparison of different extraction for the extractiom of major bioactive flavanoid compounds from spearminmt (*Mentha spicata* L.) leaves. *Food and bioproducts Engineering, 89*:67-72.
11. Krishnaiah, D., Devi, T., Bono,A. and Sarbatly, R. (2009). Studies on phytochemical constituents of six Malaysian medicinal plants, J. Med. Plants Res, 3(2): 67-72.
12. Junji, Terao., Yoshichika, Kawai., Kaeko, Murota., (2008). Vegetable flavonoids and cardiovascular disease. *Asia Pac J Clin Nutr: 17* (S1): 291-293.
13. Jegathambigai, R. (2006). Phytoestrogens (Genistein and diadzein): Distribution in tempeh and their endocrine-modulating effects in mice. M. Med.Sc. thesis, University of Malaya, P- 35-60.
14. Harborne, J.B. (1996). Chemotaxonomy of anthocyanins and phytoalexins in the Compositae. In: Hind, D.J.N., Boentje, H.J. (Eds.), Compositae: Systematics. Royal Botanic Gardens, Kew, pp. 207-218.
15. Carmen, Gherman., Monica, Culea., O, Cozar. (2000). Comparative analysis of some active principles of herb plants by GC/MS. Talanta, 53, 1:10-253-262.
16. Jegathambigai, R., Iekhsan Othman., Normadiah, M. K. and Kumar P. (2011). Effect of Isoflavones, Genistein and Diadzein on the Ovaries of Neonatal Mice. J o u r n a l o f Ecobiology, 28 (1) 1-7.
17. Tognolini., E. Barocelli., V. Ballabeni., R. Bruni., A. Bianchi, M. Chiavarini., M. Impicciatore.(2006). Comparative screening of plant essential oils: Phenylpropanoid moiety as basic core for antiplatelet activity. Life Sciences 78 1419 – 1432.
18. Sultana, Bushra., Farooq Anwar and Muhammad Ashraf. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. Molecules, 14, 2167-2180.
19. Abdille, M.H., Singh, R.P., Jayaprakasa, G.K., Jens, B.S. (2005). Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chem., 90*, 891-896.
20. Filomena Conforti Mariangela Marrelli Colica Carmela Federica Menichini Perri Valentina , Dimitar Uzunov , Giancarlo A. Statti , Pierre Duez Francesco Menichini. (2011). Bioactive phytonutrients (omega fatty acids, tocopherols, polyphenols), in vitro inhibition of nitric oxide production and free radical scavenging activity of non-cultivated Mediterranean vegetables. *Food Chemistry 129* 1413–1419.
21. Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar. (2008). Biological effects of essential oils-A review. Food and Chemical Toxicology. 46: 446-475.
22. Hussain, A. I., F. Anwar, S. T. H. Sherazi and R. Przybylski. (2008). Chemical composition, antioxidant and antimicrobial activities of basil (Ocimum basilicum) essential oils depends on seasonal variations. Food Chemistry 108:986-995.
23. Anwar, F., M. Ali, A. I. Hussain and M. Shahid. (2009). Antioxidant and Antimicrobial activities of essential oils and extracts of fennel (Foeniculum vulgare Mill.) seeds from Pakistan. Flavour and Fragrance Journal. 24: 170-176.
24. Yadegarinia, D., Gachkar, L., Rezaei, M.B., Taghizadeh, M., Astaneh, S.A and Rasooli,I. (2006). Biochemical activities of Iranian Mentha piperita L. and Myrtus communis L. essential oils Phytochemistry 67: 1249-125.
25. Dallüge, J., Van Rijn, M., Beens, J., Vreuls, R.J.J. & Brinkman U.A.Th. (2002). Comprehensive two-dimensional gas chromatography with time-of-flight mass Spectrometric detection applied to the determination of pesticides in food extracts. Journal of Chromatography A, Vol. 965, No. 1-2, pp. 207-217.
26. Gogus, F., Ozel, M.Z. & Lewis A.C. (2006). Analysis of the volatile components of cheddar cheese by direct thermal desorption – GC×GC-TOF/MS. Journal of Separation Science, Vol. 29, No. 9, pp. 1217-1222.
27. Guilhaus, M. (1995). Principles and Instrumentation in Time-of-flight Mass Spectrometry. Physical and Instrumental Concepts. Journal of Mass Spectrometry, Vol. 30, No. 11, pp.1519-1532.
28. Griffiths, G., Trueman, L., Crowther, T., Thomas, B., Smith, B. (2002). Onions-a global benefit to health. Phytotherapy Research 16, 603–61
29. Halliwell B. (2007). "Dietary polyphenolics: good, bad or indifferent for you health?" Cadiovascular Research, 73, 2. 341-347.
30. Dufresne, C.J. and Farnworth, E.R. (2001). A review of latest research findings on the health promotion properties of tea, J Nutr Biochem 12 404–421.
31. Damianaki, A., Bakogeorgou. E., Kampa, M., Notas, G., A. Hatzoglou., S. Panagiotou., C. Gemetzi.,E. Kouroumalis., P.M. Martin., E. Castanas. (2000). Potent inhibitory action of red wine polyphenol on human breast cancer cells, J Cell Biochem 78 429–441.
32. Wannee, R. Kusamran., Anong Tepsuwan., Piengchai Kupradinun. (1998). Antimutagenic and anticarcinogenic potentials of some Thai Vegetables, Mutation Research 402 ,. 247–258.
33. Jőrg, Wittig., Markus, Herderich., Eva, Ulrike, Graefe., Markus, Veit. (2001). Identification of quercetin glucuronides in human plasma by HPLC-tandem mass spectrometry. Journal of chromatography B, 753: 237-243.
34. Rajalakshmi, P.V., Kalaiselvi Senthil, K. (2009). Direct HPLC analysis of quercetin in exudates of Abutilon Indicum (Linn) Malvaceae. Journal of Pharmaceutical Science and Technology, Vol 1 (2): 80-83.
35. Wittig, J., Herderich, M., Graefe, E.U., Veit, M. (2001). Identification of quercetin glucuronides in human plasma by high- performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 753: 237–243 l., 2001).
36. Koo and Suhaila Koo Hui Mien and Suhaila Mohamed. (2001) Flavonoid (myricetin, quercetin, kaempferol, Luteilin and apigenin) content of edible tropical plants. *Journal of Agricultural and food chemistry. 49(6):* 3106-3112.
37. Hertog, M.G.L., Hollman, P.C.H., and Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J. Agric. Food Chem. 40, 1591-1598.
38. Leighton, T., Ginther, C., Fluss, L., Harter, W.K., Cansado, J., Notaro, V. (1992). Molecular characterization of quercetin and quercetin glycosides in Allium vegetables and their effects on malignant cell transformation. In: Hung, M., Ho, C.L.,Lee, C.Y. (Eds.), Phenolic Compounds from Food and their Effect on Health, vol.507. American Chemical, Society Symposium Series, pp. 220–238.
39. Junji, Terao., Yoshichika, Kawai., Kaeko, Murota., (2008). Vegetable flavonoids and cardiovascular disease. *Asia Pac J Clin Nutr: 17* (S1): 291-293.
40. Crozier, A., Jensen, E., Lean, M.E.J., McDonald. M.S. (1997). J. Chromatogr. A716 315.
41. Holl man Hollman, P. C. H.; Van Trijp, J. M. P.; Buysman, M. N.C. P.; Gaag, M. S. v. d.; Mengelers, M. J. B.; De Vries, J. H. M.; Katan, M. B. (1997). Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Lett.*, *418*, 152-156.
42. Gary Williamson., Geoff W. Plumb., Yasushi Uda., Keith R. Price and Michael JC Rhodes.(1996). Dietary querc etin glycoside: antioxidant activity and induction of the anticarcinogenic phase II marker enzyme quinine reductase in Hepalclc7 cells. Carcinogenesis vol 17: 11, 2387-2387
43. Goet, R. K., Pandey, V. B., Dwivedi, S. P. D., Rao, Y. V. (1988). Anti-inflammatoand antiulcer effects of kaempferol, a flavone, isolated from Rhamnus procumbens. Indian J. Exp. Biol. 26, 121-124.
44. Nothlings, U., Schulze, M. B., Weikert, C., Boeing, H., van der Schouw, Y. T., Bamia, C., et al. (2008). Intake of vegetables, legumes, and fruit, and risk for all-cause, cardiovascular, and cancer mortality in a European diabetic population. The Journal of Nutrition, 138, 775−78.
45. Xu, B. J., & Chang, S. K. C. (2007). A comparative study on phenolic profiles and antioxidant activities of legumes as affected by extraction solvents. Journal of Food Science,72, s159−s166.
46. Angeloni, C., Spencer, J. P. E., Leonine, E., Biagi, P. L., & Hrelia, S. (2007). Role of quercetin and its in vivo metabolites in protecting H9c2 cells against oxidative stress.
47. Sperker, B., Werner, U., Murdter, T.E. et al. (2000). Expression and function of beta-glucuronidase in pancreatic cancer: potential role in drug targeting. Naunyn Schmiedebergs Arch Pharmacol ;362:110–5.
48. Claudine Manach., Augustin Scalbert., Christine Morand., Christian Rémésy and Liliana Jime´nez.(2004) Polyphenols: food sources and bioavailability1,2. Am J Clin Nutr;79:727–47.