IOP Conf. Series: Materials Science and Engineering **263** (2017) 022018 doi:10.1088/1757-899X/263/2/022018

Preliminary phytochemical analysis, Antioxidant and cytotoxicity test of *Carissa edulis* Vahl dried fruits

J Fowsiya and G Madhumitha
Chemistry of Heterocycles & Natural Product Research Laboratory, Department of Chemistry, School of Advanced Sciences, VIT University, Vellore, 632 014, India

E-mail : Dr.g.madhumitha@gmail.com; madhumitha.g@vit.ac.in

Abstract. Plants are the main source of medicine which is used in traditional as well as modern medicine in recent years for curing many diseases. *Carissa edulis* Vahl is one of the traditional plants which have healing property on diarrhea, toothache and chest pain. The present work aims on phytochemical, antioxidant and *in vitro* cytotoxicity test of *C. edulis* dried fruits. The different solvent extracts obtained from petroleum ether, ethyl acetate, chloroform, ethanol and water have been evaluated the presence of phytochemicals. Several assays were carried out like total antioxidant, DPPH, reducing power and thiobarbituric acid to investigate the free radical scavenging property. In addition, the cytotoxicity study also carried out on human lung cancer cells (A549). Among different solvent extract, ethanol exhibited strong antioxidant activity. Additionally, the *in vitro* cytotoxicity test of *C. edulis* on human lung cancer cell (A549) showed IC_{50} value 405.704 ± 2.42 µg/mL. Therefore, *C. edulis* could be useful as a potential preventive intervention for free radicals mediated diseases as well as an antioxidant drug in the pharmaceutical industry.

1. Introduction

The many numbers of medicinal plants are utilized for diabetes, obesity, skin diseases etc., The free radicals inside the body play important role in cellular damage and development of many diseases which could be stabilized using antioxidant [1, 2]. The increased level of free radical cause oxidative stress, protein damage, coronary artery disease, hypertension, diabetes and metabolic syndrome. Antioxidants are chemical substances has the capacity to inhibit free radicals which occur with atmospheric oxygen or reactive oxygen species [3]. The antioxidants can classify into endogenous antioxidants and exogenous antioxidants. The endogenous radical scavengers are enzymes such as catalase, glutathione peroxidase or non-enzymatic chemicals such as uric acid, albumin and bilirubin. The exogenous antioxidants arise from natural sources and synthetic compounds like butylhydroxyanisole. In the recent years, natural sources have gained a potential application towards free radical inhibition than the synthetic drug which causes side effects [4]. The natural sources are rich in vitamins, phenolic compounds, flavonoids, alkaloids, lignans, etc., which can act as an antioxidant in oxidative process [5, 6]. The natural sources such as blackberries, walnuts, strawberries, grapes, etc., are a few examples which have high antioxidant [7-14].

The *Carissa edulis* (*C. edulis*) is one of the traditionally used plants which belong to Apocynanceae family and used to cure tooth decay, the risk of depression and skin premature. The aerial part of the *C. edulis* contains vitamin C, B1, B2, A, calcium, iron, magnesium and potassium. The fresh fruits were used to make jam, vinegar, soups and root bark used to cure body pain, gastric problem, diuretic

and malaria [15]. Many reports confirmed the presence of terpenes, flavonoids, phenolic compounds, glycosides, and lignan. The most used part root contains rutin, pinitol, amyrin, lupeol, carissin, carissone etc., [16]. The root also used for biological activities including, antimicrobial, antiviral, anti-inflammatory and anticonvulsant activity. The *C. edulis* root tea has a high content of phenolic compounds namely oleuropein and lupeol which is against Herps simplex virus disease. These phenolic compounds are present in high content in the *C. edulis* tea than any other herb [17, 18]. Therefore, there is no report on the dried fruit of *C. edulis* for its phytochemcial analysis and biological activity. In this study dried fruit of *C. edulis* has been utilized for its phytochemical, antioxidant, and cytotoxicity test.

2. Experimental section

2.1. Microwave assisted extraction

The fresh fruit of *C. edulis* was procured from Vellore local market and authenticated (PARC/2014/2321). The dried fruits were powdered and subjected for extraction in microwave extractor at 400 W, at 60 º C for 30 min. Petroleum ether, ethyl acetate, chloroform, ethanol and water were used for extraction.

2.2 Phytochemical analysis

The different solvent extracts were utilized for determination of qualitative and quantitative investigation of phytochemicals. The quantitative and qualitative analysis was carried out as per the standard protocol with slight modifications.

2.2.1 Qualitative analysis of Phytoconstituents

The extracts are taken for phytochemical analysis to study the presence of phenolic, flavonoids, glycosides, saponin, alkaloids and terpenoids [19-22].

2.2.2 Total Phenolic content

The extract of *C. edulis* was taken for total phenolic compounds by Folin-Ciocalteau (FC) method [23-26]. Different concentrations of extracts (mg/ml) 100-500 µL/mg were added to 2.5 ml of 1/10 diluted FC and sodium carbonate (2 mL). The test solution was kept in incubator at 45 °C for 15 min and the absorbance has been monitored at 765 nm. The obtained results expressed in mg of gallic acid equivalents per mg of extract.

2.2.3 Determination of total flavonoids

About 100-500 µL of ethanol extract was diluted by adding distilled water and 75 µL of sodium nitrite (5%). Aluminum chloride (150 µL of 10%) and NaOH (0.5 mL of 1 M) also added and diluted with distilled water. The absorbances were recorded at 510 nm and quercetin was taken as a standard. The obtained results expressed in mg of quercetin equivalents per mg of extract [27, 28].

2.2.4. Total alkaloids determination

The crude extracts were further mixed with dil.HCl followed by chloroform extraction. The pH of the aqueous layer adjust to neutral with NaOH and 5 ml of Bromocresol green and 5 ml of phosphate buffer were added drop wise. The aqueous layer again extracted with chloroform. The both chloroform and aqueous layers were taken for the determination of total alkaloids [29-31].

2.3. In vitro antioxidant activity

2.3.1. Total antioxidant capacity (TAC)

The various solvent extracts of C. edulis in absolute ethanol were added to 1 mL of reagent solution (0.6 M H_2SO_4, 28 mM Na_3PO_4 and 4 mM $(NH_4)_6Mo_7O_{24}$). The mixture kept at 95 ºC for incubation and placed at room temperature to become cool. The absorbance of the mixture was monitored at 695

nm using ascorbic acid as a standard. TAC was calculated using standard curve and expressed in terms of mg of standard [32-35].

2.3.2 DPPH scavenging assay

About 100-500 µL concentration of Petroleum ether, ethyl acetate, chloroform and ethanol extract were prepared in 99.9 % absolute ethanol (1mg/mL) and added to 3 mL of DPPH (0.1 mM) [36-39]. The solutions were set aside in dark condition about to 30 min and the absorbance was measured at 517 nm. Ascorbic acid (1 mg/mL) was taken as standard solution in 100-500 µL concentrations and it was added with 3 mL of DPPH solution. The test solution was monitored as per spectroscopic method and the % inhibitions were calculated using equation **(1)**.

$$\textbf{\% Inhibition} = [A_c - A_s / A_c] \times 100 \quad \cdots\cdots\cdots\blacktriangleright (1)$$

Control absorbance denoted by A_c and sample absorbance denoted by A_s.

2.3.3 Thiobarbituric acid assay

The different solvent extracts were varied from 100-500 µL concentration added to the mixture of 2 mL of 20 % of trichloroacetic acid and 2 mL of 0.67 % of thiobarbituric acid solution. The test solution was boiled at 60 ºC for 10 min [40-42]. The solutions were centrifuged at 3500 rpm for 30 min and the absorbance of test solution and the standard ascorbic acid was monitored at 532 nm by UV-Visible spectrophotometer. Total inhibition of the extracts was calculated using equation **(1).**

2.3.4 Hydrogen peroxide assay

The capability of the *C. edulis* extract to inhibit hydrogen peroxide radical was determined by following method. About 3.4 mL of hydrogen peroxide in phosphate buffer and 0.6 mL H_2O_2 (40 mM) was added with extracts. The scavenging property of hydrogen peroxide was determined using absorbance at 230 nm [43, 44]. The inhibition was calculated using equation **(1).**

2.3.5 Reducing power assay

About 100-500 µL of extracts have been added to phosphate buffer (0.5 mL of 0.2 M, pH 6.6) and 0.5 mL of 1% $K_3[Fe(CN)_6]$ mixture. The solutions placed in incubator at 60 °C for 30 min followed by centrifugation with 0.5 mL of 10 % trichloro acetic acid. The obtained supernatant added to 1 mL of distilled water and 0.2 mL of 0.1 % ferric chloride hexahydrate. The color change was monitored after 10 min at 700 nm using ascorbic acid as a standard solution [45-50]. The inhibition was calculated using equation **(1).**

2.4. Gas chromatography mass spectroscopy (GC-MS) analysis of C. edulis

The GC-MS study was used for identification of the secondary metabolites which are present in the ethanol extract of *C. edulis.* The ethanol extract was analyzed by 680 Perkin Elmer gas chromatography with Elite-5 capillary column consist of 5% Diphenyl 95% dimethyl poly siloxane.

2.5. In vitro cytotoxicity test

The ethanol extract of *C. edulis* was tested for In vitro cytotoxicity using Lung cancer cells (A549) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay followed by standard protocol with slight modificatios [51-53]. The A549 cells and *C. edulis* extract (50, 100, 200, 400, 600 µg/mL) was poured in 96-well plates containing a final volume of 100 µL/well. Further, it was incubated and added to 10 µL of MTT per well to achieve the final concentration of 0.45 mg/mL. After 24 h of incubation at 37 ºC, 100 µL solubilization solutions added to each well to dissolve formazan crystals. The absorbance was recorded at 570 nm and inhibition was calculated using the following equation,

$$\% \text{ cell inhibition} = 100 - [(A_t - A_b)/(A_c - A_b)] \times 100$$

Where, A_t, A_b and A_c indicate the absorbance of tested sample, blank and control.

3. Results

3.1 Qualitative and quantitative analysis of phytochemicals of C. edulis

The phytochemical test of various solvent extracts from dried fruits of *C. edulis* has been evaluated and shown in Table 1. The preliminary phytochemical analysis showed a positive result for diterpene, terpenoids, carbohydrates, amino acids and resins in petroleum ether extract. The ethyl acetate extract contains flavonoids, glycosides, phenolics, phytosterol, diterpene, terpenoids, anthocyanin, carbohydrates, amino acids and resins. The water and ethanol extracts showed the presence of alkaloids, flavonoids, coumarin etc. Table 2 shows the amount of phenolic and flavonoids present in ethyl acetate, chloroform, ethanol and water extracts.

Table 1 Preliminary phytochemical analysis of dried fruits of *Carissa edulis*

Phytochemicals	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Water
Alkaloid	-	-	-	+	+
Flavonoid	-	+	+	+	+
Glycoside	-	+	-	+	-
Saponin	-	-	-	-	-
Phytosterol	-	+	-	-	-
Polyphenol	-	+	+	+	+
Tannin	-	+	-	+	-
Diterpene	+	+	-	+	-
Terpenoid	+	+	-	+	-
Anthocyanin	-	+	-	+	+
Coumarin	-	+	-	+	+
Lignin	-	-	-	-	-
Carbohydrate	+	+	+	+	+
Protein/amino acid	+	+	+	+	+
Oil and fat	-	-	-	-	-
Gum and Mucilage	-	-	-	-	-
Resin	+	+	+	+	+

Table 2 Total phenolic, flavonoids and alkaloids of *C. edulis*

Carissa edulis extracts	Total phenolic content (mg GAE/mg of extract)	Total flavonoids (mg QE/ mg of extract)	Total alkaloids (mg Atropine/ mg of extract)
Petroleum ether	-	-	-

Ethyl acetate	28.33±0.02	29±0.5	-
Chloroform	37.37±0.03	35±1.2	-
Water	94.97±0.22	50±2.1	19.6±0.001
Ethanol	96±0.5	70±0.7	21.33±0.001

Due to the low polarity of petroleum ether, it doesn't show the presence of phenolic and flavonoids. Therefore, the petroleum ether extracts not taken to determine total phenolic and flavonoids. The quantitative phytochemical analysis showed a significant content of phenolic and flavonoid in all extracts. Among all the solvent extracts, ethanol extract has a higher content of phenolic, flavonoids and alkaloids than other extracts. The phenolic and flavonoids are responsible for most of the biological activities. In conclusion, the phenolic and flavonoids content are in the following order: Ethanol > Water > Chloroform > Ethyl acetate. The ethanol extract has a higher content of phenolic, flavonoids and alkaloids when compared with a standard curve (Figure1-4). The ethanol extract has 96±0.5 mg of the gallic acid equivalent of phenolic content. Table 2 shows the quantitative analysis of phenolic, flavonoids and alkaloids. The phenolic content of, ethyl acetate, chloroform, water and ethanol extracts were found to be 28.33±0.02, 37.37±0.03, 94.97±0.22 and 96±0.5 respectively. The alkaloid content was 19.6±0.001 for water extract and 21.33±0.001 for ethanol extract as expressed mg of atropine equivalent. Accordingly, the phenolic and flavonoids are good responsible agents for the antioxidant activity by donating an electron.

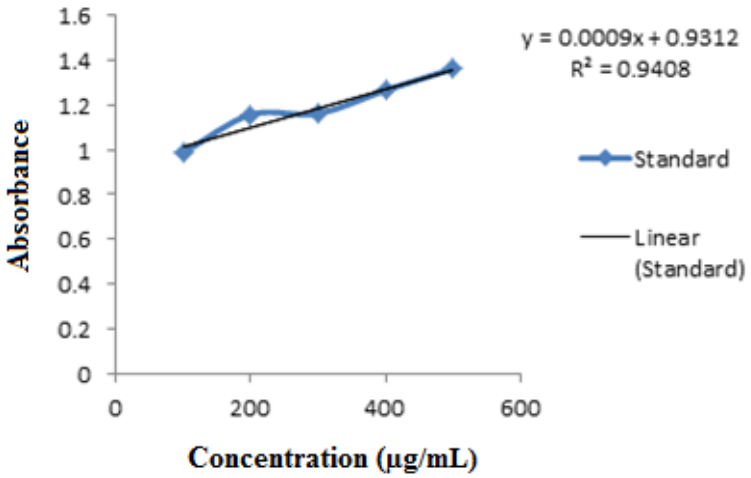

Figure 1. Standard curve for total phenolic of *C. edulis*

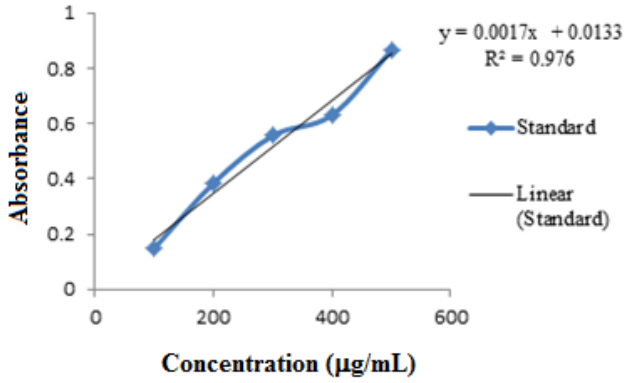

Figure 2. Standard curve for total flavonoids

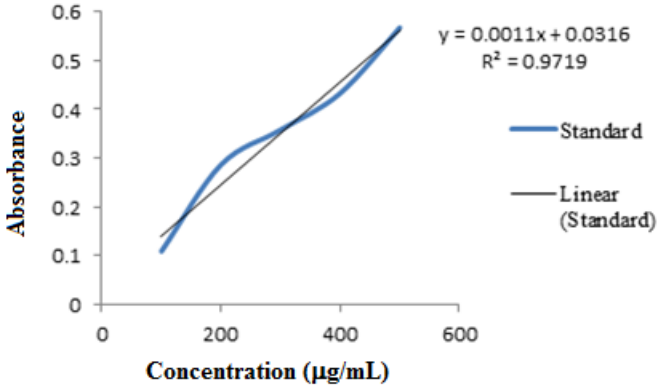

Figure 3. Standard curve for total alkaloids

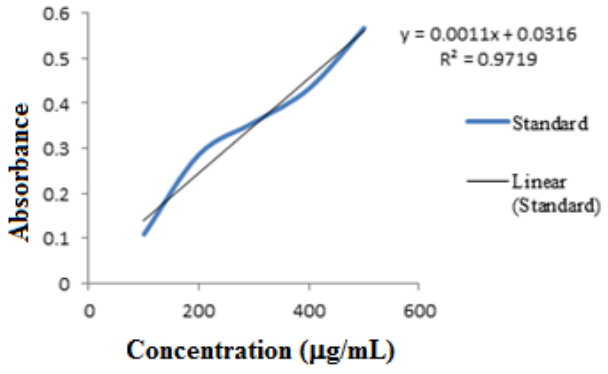

Figure 4. Standard curve of total antioxidant capacity

3.2 In vitro antioxidant activity

3.2.1 Total antioxidant activity

TAC of extracts was calculated using linear equation y= 0.0011x+0.0316, R^2=0.9719. The ethanol extract has higher antioxidant capacity compared with other extract. Where "y" is an absorbance at 695 nm and "x" is the concentration of ascorbic acid equivalent µg/mL (Figure 5 and 6). The TAC was determined by reduction of phosphomolybdenum complex. In the typical mechanism, Mo^{4+} to Mo^{5+} was reduced and formed a green color Mo^{5+} complex in acidic pH condition [54]. The phosphomolybdenum assay is a direct method to evaluate the reducing efficiency of antioxidant. Unlike other methods, it is unique and forms a phosphomolybdinum complex without generation of free radicals. Therefore, this method is different from the i*n-vitro* antioxidant methods.

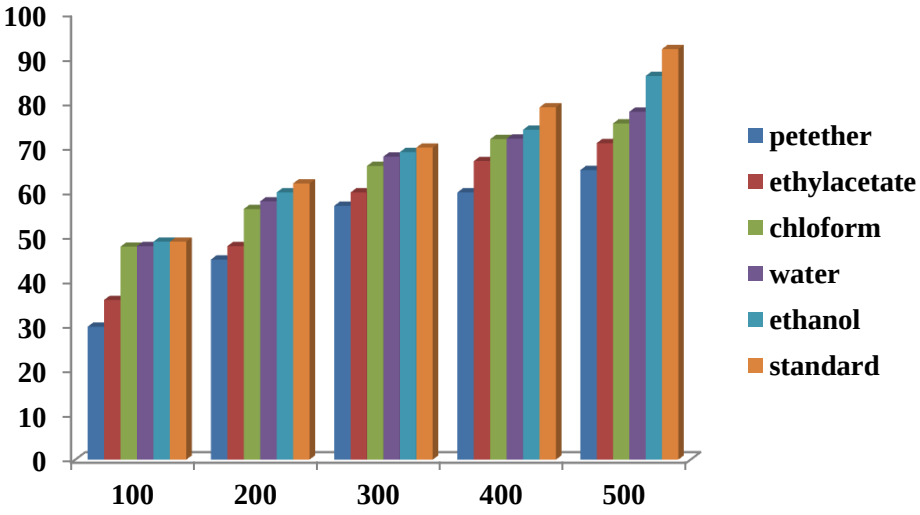

Figure 5. Total antioxidant capacity of *C. edulis*

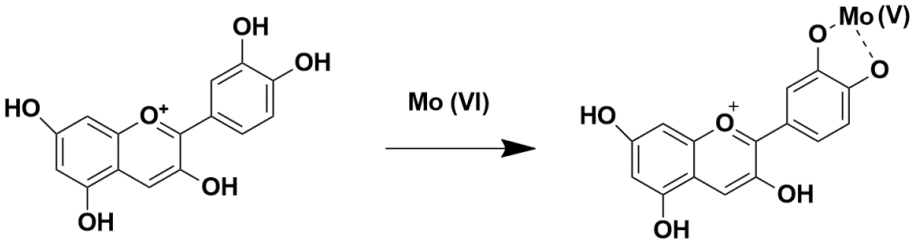

Figure 6. Mechanism of total antioxidant activity

3.2.2 Inhibition of DPPH radical

The *C. edulis* extracts were studied for the antioxidant activity using DPPH. The DPPH radical inhibition percentage is shown in Table 3. The ethanol extract showed good free radical inhibiting efficiency when compared to that of petroleum ether, ethyl acetate, chloroform and water extracts. The ethanol extract has high inhibition around 80.48 % at 500 µg/mL (IC_{50} value 169.98±0.03). The IC_{50} values were found to be 488.05±0.03 for petroleum ether, 330.98±0.003 for ethyl acetate, 298.05±0.01 for chloroform and 195.4±0.05 for water extract. The less IC_{50} values used to represent the inhibition of 50% of free radicals and if it is less, the inhibition will be more. Therefore, the IC_{50} value of ethanol extract was very less when compared to other solvent extracts. Hence, *C. edulis* act as a good free radical scavenger at low concentration. As the concentration increases, the inhibition of ethanol extract was found to be almost equal to that of the standard.

Table 3 DPPH scavenging activity of *C. edulis*

Concentration (µg/mL)	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Water	Standard
100	19.25±0.5	20.98±0.0	30.26±0.3	45.72±0.2	41.39±0.1	48.48±0.9
200	23.35±0.01	30.04±0.1	40.56±0.5	56.33±0.1	54.01±0.1	79.49±1.2
300	31.81±0.03	52.8±0.5	43.4±0.7	59.89±0.1	59.62±0.1	80.23±0.8
400	41.39±0.00	53.34±0.2	60.21±0.2	62.43±0.1	61.85±0.3	80.56±0.1
500	52.59±0.01	72.51±0.1	76.73±0.1	80.48±0.1	76.06±0.1	82.57±0.14
IC50	**488.017**	**330.507**	**298.051**	**169.980**	**195.429**	**99.225**

3.2.3 Thiobarbiutric acid assay

The *C. edulis* fruit showed less antioxidant activity at lower concentration and significant effect at higher concentration. The percentage inhibitions of the activity have been given in Table 4. The ethanol and water extract shows effective inhibition of radicals than other extracts. The IC_{50} values were 284.92±0.1, 230.97, 114 and 106.09 µg/mL for petroleum ether, ethyl acetate, chloroform and water extracts respectively. The less IC_{50} was 94.83 µg/mL for ethanol extract of *C. edulis*. All the extract taken for the assay has dose-dependent activity towards radicals.

Table 4 Thiobarbeutric acid assay (%Inhibition)

Concentration (µg/mL)	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Water	Standard
100	30.01±1.5	36±1.0	35.12±0.12	48±0.5	47.9±0.3	50.1±0.01
200	45.01±0.51	48±0.2	48.31±0.31	58±0.9	56.3±0.15	60±0.05
300	57.08±0.23	60±0.05	94.56±0.6	69±0.0	65.9±0.34	69±0.5
400	60.98±0.13	67±0.05	95.83±0.3	72±0.0	71.4±0.11	74±0.2
500	65.90±0.61	71±0.15	96.88±0.1	78±0.2	75.4±0.0	86±0.1
IC50	**284.921**	**230.974**	**185.992**	**106.190**	**114.532**	**94.839**

3.2.4 Hydrogen peroxide assay

Table 5 shows H_2O_2 scavenging activity of the various extracts of *C. edulis*. All the extracts showed a dose-dependent fashion on free radical scavenging ability. The ethanol extract exhibited stronger scavenging capacity similar to the previous assay. The investigation on the different solvent extraction, the peroxide inhibition was as in the following order, Petroleum extract <ethyl acetate < chloroform < water < ethanol. Among all the IC_{50} values of extracts, ethanol has less IC_{50} value as 94 µg/mL which inhibits the 50% of inhibition at low concentration. The IC_{50} values were 284.92 µg/mL for petroleum ether extract and 230.97 µg/mL for ethyl acetate extract. The highly polar solvent such as chloroform and water has 114 and 106 µg/mL of IC_{50} values.

Table 5 Hydrogen peroxide scavenging activity of *C. edulis*

Concentration (µg/mL)	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Water	Standard
100	21.75±1.5	27±1.0	42.92±0.12	45±0.52	44.0±0.12	46.25±0.01
200	32.5±0.51	33.75±0.2	49.62±0.31	57±0.0	52.1±0.13	57.5±0.15
300	36.26±0.23	43.75±0.05	52.5±0.6	68±0.0	60.0±0.04	68.75±1.5
400	41.25±0.13	60.5±0.05	60±0.3	70±0.0	66.0±0.13	77.5±1.2
500	50.1±0.61	68.75±0.15	71.25±0.1	72±0.2	71±0.0	82.5±1.1
IC50	**503.196**	**328.87**	**224.9769**	**138.113**	**174.828**	**125.196**

3.2.5 Reducing power assay

The ethanol and water extracts have shown good reducing power than other extracts of *C. edulis.* The assay was determined for the different concentration of extracts like 100, 200, 300, 400 and 500 µg/mL. The reducing power of petroleum ether extract at low concentration (100 µg/mL) was negligible. The ethyl acetate and petroleum ether extract showed a less reducing capacity against iron (III). The color of the solution of reducing power assay turns yellow to shaded blue and green due to the presence of various phytochemicals in extracts. The IC50 values were found as 239, 224, 128, 109 and 101.41 µg for petroleum ether, ethyl acetate, chloroform, water and ethanol extracts respectively (Table 6). The reducing power assay has been calculated by the ability of *C. edulis* extract in the reduction of the iron (III) into iron (II) complex.

Table 6 Reducing power of *C. edulis*

Concentration (µg/mL)	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Water	Standard
100	36.1±1.0	36.0±1.2	35.12±0.1	48.0±0.14	47.9±0.15	49.0±0.53
200	45.0±0.0	50.0±0.4	48.31±0.5	58.1±0.0	54.0±0.05	60±0.12
300	59±0.05	60±0.25	94.56±0.0	68.0±0.04	65.9±0.12	70.3±0.45
400	63±0.06	67.0±0.10	95.88±0.5	72.0±0.10	69.0±0.65	74.6±0.33
500	68±1.5	72±0.56	97.89±0.0	80±0.0	76.0±0.45	86.2±0.12
IC50	**239.68**	**224.393**	**185.992**	**109.020**	**128.503**	**101.419**

3.3 Gas chromatography mass spectroscopy analysis (GC-MS) of C. edulis

The crude ethanol extract showed fourteen peaks in the GC-MS chromatogram (Fig.ure 7) which are identified according to their retention time on fused silica capillary column. The identified compounds include hydrocarbon, fatty acids, flavonoids and ketones (Table 7). The α-D-glucose, Lupeol and Diethyl phthalate was identified as a major chemical constituent (36.535 %, 18.109 % and 13.615 %).

Table 7 GC-MS analysis of *C. edulis*

S.No	RT	Area %	Chemical name
1	7.245	0.562	Butanoic acid
2	14.393	13.615	Diethyl phthalate
3	15.053	12.311	2-Undecene
5	16.854	36.535	*α-D*-glucose
6	20.290	0.454	Propanoic acid
7	23.512	0.649	14-heptadecenal
8	25.197	2.322	Pentadecenal
11	31.380	18.109	Lupeol

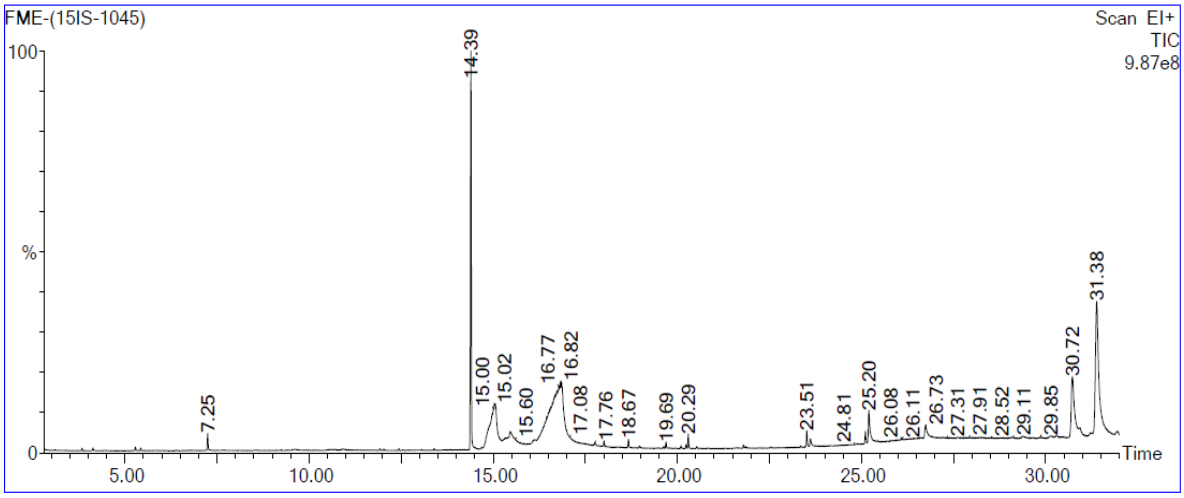

Figure 7. GC-MS chromatogram of ethanol extract of *C. edulis*

3.4. In vitro cytotoxicity test

The determination of cytotoxicity by ethanol extract of *C. edulis* was performed using MTT assay. The results showed that increase of extract concentration increases the number of dead cells. The ethanol extract treatment of A549 cells were found to be significantly decreased the cell viability in a dose depended manner at 28 h incubation (Figure 8). The IC50 values were found to be 405.704 ± 2.42 µg/mL. The morphological observation for MTT reduction to purple color formazan was shown in Fig.ure 9.

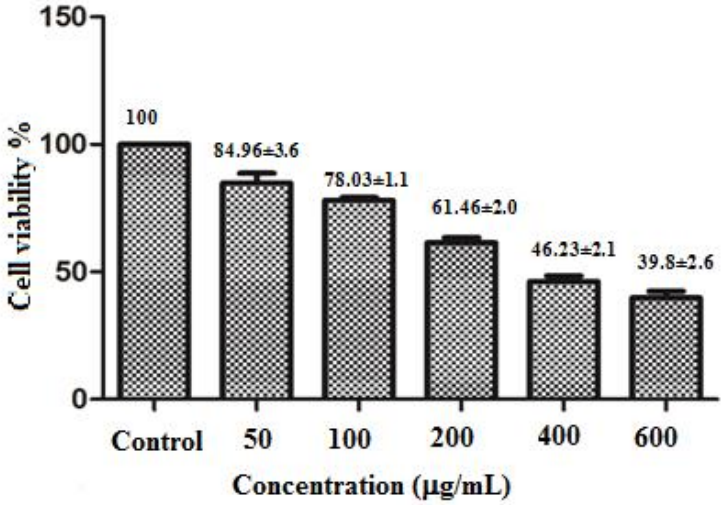

Figure 8. Percentage cell viability of A549 cells using *C. edulis*

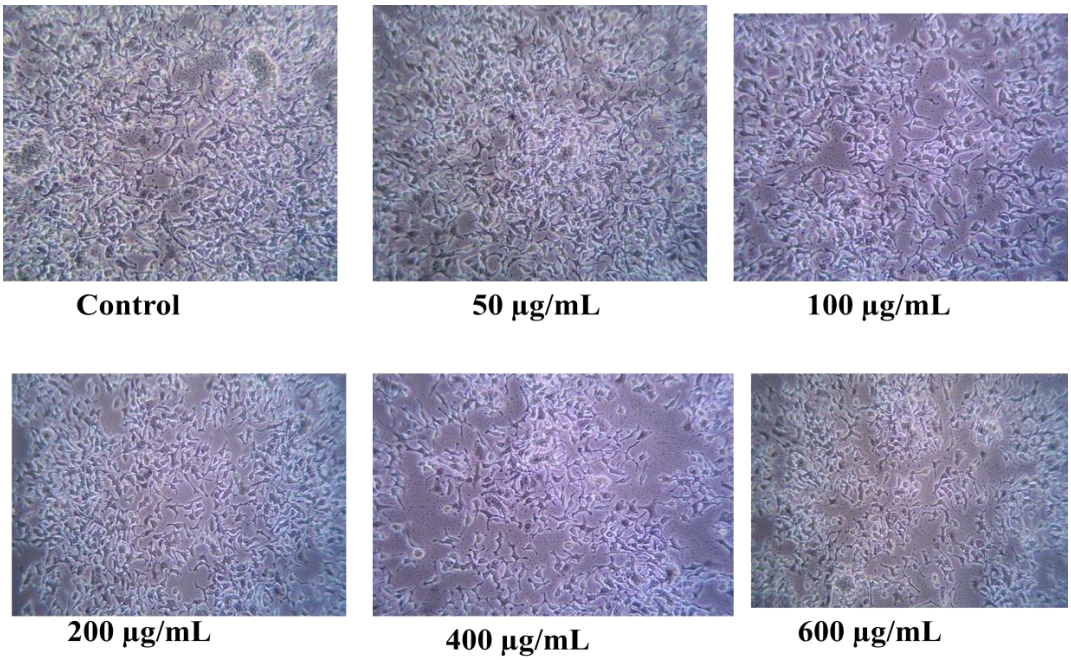

Figure 9. Lung cancer cells treated with *C. edulis*

4. Discussion

The presence of phytochemicals in *C. edulis* has played an important role in antioxidant activity. The quantitative and qualitative analysis showed the phytochemicals content in the extract. These phytochemicals have the capacity to inhibit the free radical significantly. The 1, 1-diphenyl-2-picrylhyrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) one of the stable radical delocalized of a spare electron on the molecule and it cannot dimerize further and it acts as free radical forms a deep violet color [55]. From the extracts, it takes a hydrogen atom and forms non-radical which induces the violet

color (Figure 10). The phenolic and flavonoids of *C. edulis* can donate hydrogen to the synthetic stable free radical DPPH to change it as non-reactive species. The previous reports on *C. edulis* root state that 62.7 % of inhibition against DPPH radical [56] whereas fruit has 80.48 ± 0.1% of inhibition by ethanol extract. The variations among the natural plants lead to the difference in the antioxidant activity. Therefore, higher the phenolic and flavonoids content in the extract can lead to the high inhibition of free radicals [57].

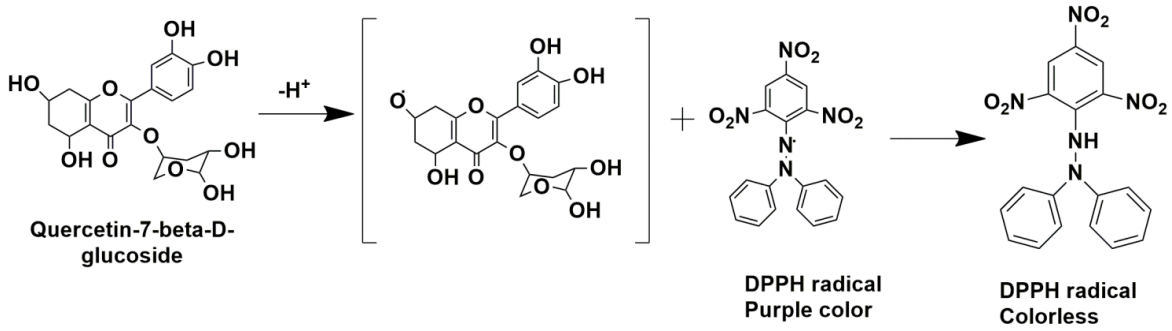

Figure 10. DPPH scavenging mechanism

The thiobarbituric acid assay that was proposed as the formation of adducts among the Thiobarbituric acid (TBA) and Malondialdehyde (MDA) (Figure 11). This method is not very specific and effective on reaction conditions on color development [50]. The naturally available reductants in the extract are involved in the inhibition process. The reducing capacities of phytochemicals of *C. edulis* have served a significant efficacy in the thiobarbituric acid assay. Hence, the free radicals which are occurred in human metabolism can inhibit by phytochemicals [58].

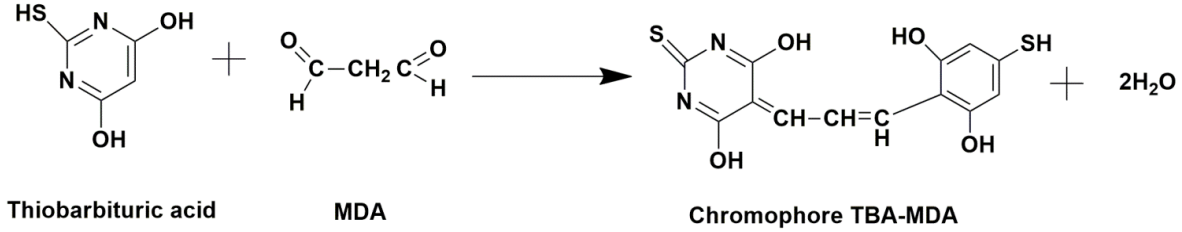

Figure 11. Adduct formation in thiobarbituric acid assay

The hydrogen peroxide decomposed into oxygen and water by producing hydroxyl (OH·) which causes lipid peroxidation and damage DNA in the human body.The superoxide anion (O2.-) produced in the reaction, protonated at low pH forms hydroperoxyl radical ·HO2 and both of these radicals undergoes spontaneous reaction to produce hydrogen peroxide and hydroxyl radical [59] (Fig.ure12). Hydrogen peroxide is less reactive than superoxide radical, but presence of any transition metals especially Fe2+ which is present in the biological system, causes the formation of hydroxyl radical by Fenton reaction [44].

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$
$$Fe^{3+} + O_2^{\cdot -} \longrightarrow O_2 + Fe^{2+}$$
$$\text{Total reaction: } O_2^{\cdot -} + H_2O_2 \longrightarrow O_2 + OH^{\cdot} + OH^-$$

Hence, the flavonoids or phenolic compounds present in the extracts can terminate the free radical long chain reaction (Figure 13).

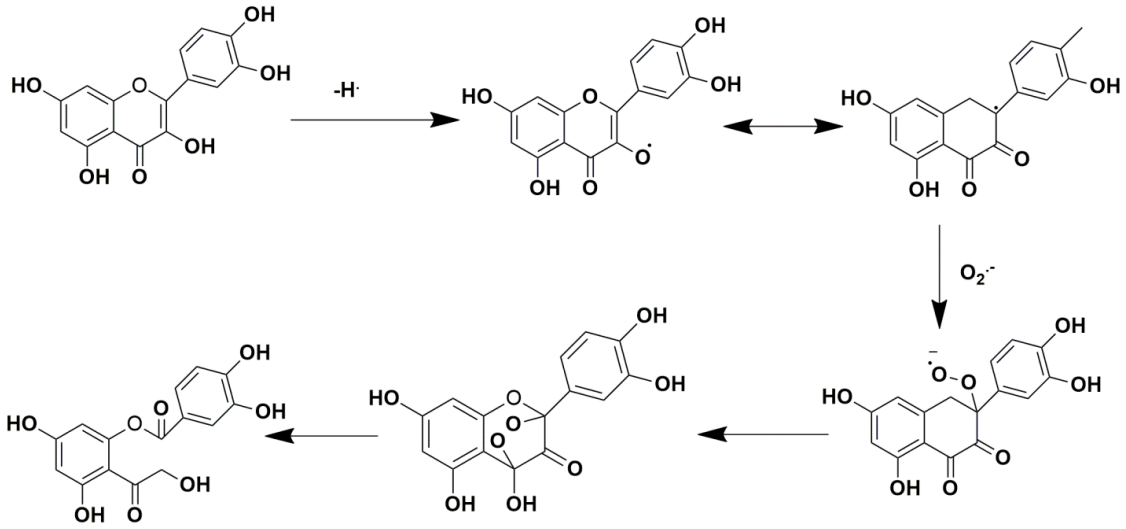

Figure 12. Superoxide radical scavenging mechanism by quercetin

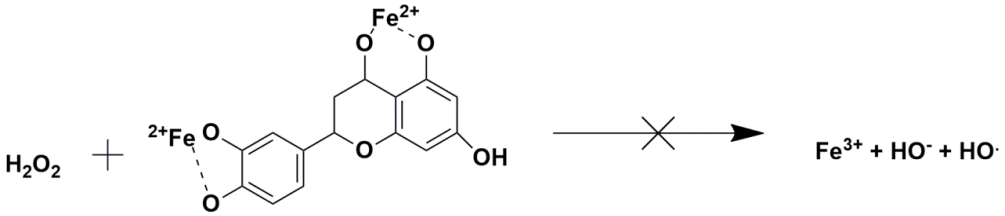

Figure 13. Termination of free radical by flavonoid

All results showed antioxidant activity in a dose dependent manner. Reducing power of all the extracts was correlated with increasing concentration. Hence, the ethanol extract pronounced more reducing capacity than other solvent extracts of *C. edulis*. The principle behind the reducing power capability is the presence of reductants which leads to the breaking of free radical chains by donating hydrogen atom. These reductants also studied for some precursors to prevent the formation of peroxide [60, 61]. The inhibition of free radicals in the entire assay, the petroleum extract has a significant scavenging activity. This may due to the presence of terpenoids or phytosterols instead of phenolic and flavonoids compounds and these kinds of bioactive compounds may quench with the Fe (III) species and reduce into Fe (II) [62-64].

Nowadays, research on the antioxidant activity of plant extracts have been achieved a great application to prevent the free radical formation and ageing problem that are caused by the abnormal diet and side effect of UV rays [65-67]. The phenolic compounds, flavonoids and alkaloids implied potential bioactive natural compounds and more studies are required regarding the phytochemical and its biological applications. Moreover, the possibility of biological effects of extract is considered due to the multi bioactive effect of phytochemicals present in extract [68]. Hence, the results from our report states that the promising antioxidant agents can be used in the pharmaceutical and cosmetic field to prevent the radical formation and it can be incorporated into photoprotective products to provide a better result in photoprotective effect due to the antioxidant efficacy of *C. edulis* fruit. The major compounds indentified by GC-MS such as Lupeol and pentadecenal may be responsible for antioxidant activity.

5. Conclusion

The results presented here constitute the first report on antioxidant activity of *C. edulis*. The various solvent extracts of *C. edulis* fruit used for antioxidant activity in a dose dependent manner. The phytochemicals such as terpenoids, phenolic compounds, flavonoids and alkaloids etc., are played important role in antioxidant activity. The qualitative and quantitative analysis of ethanol extract had good free radical scavenging activity than other extracts. Therefore, the *C. edulis* could be used as ingredients in other food products or drug to improve antioxidant capacity. The ethanol extract proved to be an effective solvent for extraction for high quantity of bioactive compounds and greater efficacy towards inhibition of radicals in all assays which we have carried out. In addition, potential efforts need to be taken in clinical rats for further biological applications. On the attention of all these results, this work may guide researcher in future in the field of phytochemistry and pharmaceutical science.

Conflict of interest

No conflict of interest.

Acknowledgment

The authors thankful to VIT University, Vellore and DST-SERB (FTYS-SB/FT/CS-113/2013) for their research support, instrument facility and financial supports.

References

[1] Chang S K, Alasalvar C, and Shahidi F 2016 *J. Func. Food.* **21** 113-132.
[2] Zhang Y J, Gan R Y, Zhou Y, Li A N, Xu DP and Li H B 2015 *Molecul* **27** 21138-21156.
[3] Martinez U O, Esparza J R and Fragoso L R 2014 *Nat. Prod. Chem. Res.* **2** 1-8.
[4] Gayatri N, Mruntyanjay S and Rajani K S 2014 *Res. J. Med. Plant.* **8** 50-81.
[5] Adam D and Carmen G C 2000 *Am. J. Clin. Nutr.* **72** 1424-1435.
[6] Dhayalan M, Jegadeeswari L and Gandhi N 2015 *Asian. J. Pharm. Clinic. Res.* **8** 11-23.
[7] Boyer J and Rui H L 2004 *Nutr. J.* **3** 1-15.
[8] Taylor J L, Rabe T, McGaw L, Jager J and Staden J 2001 *Plant. Growth. Regul.* **34** 23-37.
[9] Kazeem M I and Ashafa A O T 2015 *J. Herbal. Med.* **5** 158-164.
[10] Vuong Q V, Hirun S, Roach P D, Bowyer M C, Phillips P A and Scarlet C J 2013 *J. Herbal. Med.* **3** 104-111.
[11] Vuong Q V, Hirun S, Chuen L K, Goldsmith C D, Bowyer M C, Chalmers A C, Phillips P A and Scarlett C J 2014 *J. Herbal. Med.* **4** 134-140.
[12] Abdullah D, Yusuf U, Mukemre M, Turker M and Konczak I 2015 *J. Herbal. Med.* **5** 211-216.
[13] Kazemi M 2015 *J. Herbal. Med.* **5** 217-222.
[14] Marinova E M and Yanishlieva N V 2003 *Food. Chem.* **81** 189-197.
[15] Al-Youssef H M and Hassan H B 2014 *Int. J. Currn. Res. Chem. Pharma. Sci.* **1** 12-24.
[16] Al-Youssef H M and Hassan HB 2017 *Arabian. J. Chem.* **10** 109-113.
[17] Omino and Kokwaro 1993 *J. Ethnopharmacol.* **40**: 167-180.
[18] Festus M T, Geoffrey M R, Faith W M, Eliud N M, Wilson N, Kazuko K, Georffrey M M, Charles N M, Joseph M M, Lucia K K, Esau O and Mawuli W K 2006 *J. Ethnopharmacol.* **104** 92-99.
[19] Nguyen V T, Bowyer M C, Vuong Q V, Altena A V and Scarlett C *J. Indus. Crop. Prod.* **67** 192-200.
[20] Shettar A K, Sateesh M K, Kaliwal B B and Vedamurthy A B 2017 *South. Afr. J. Bot.* **111** 202-211.
[21] Ganguly B, Kumar N, Ahmad A H and Rastogi S K 2017 *J. Ginseng. Res.* doi.org/10.1016/j.jgr.2017.05.002.
[22] Odeyemi S, Afolayan A and Bradley G 2017*Asian. Pac. J. Trop. Biomed.* DOI: 10.1016/j.apjtb.2017.05.013.

[23] Mohammadi M, Alaei M and Bajalan I 2016 *Orient. Pharm. Exp. Med.* **16** 31-35.
[24] Lellau T F and Liebezeit G 2001*Sencken. Maritima.* **31** 1-9.
[25] Saeed N, Khan M R and Shabbir M 2012 *BMC. Compl. Alt. Med.* **12** 1-12.
[26] Xiaoming L, Ningyang L, Qiao X, Qiu Z and Liu P 2017 *J. Food. Drug. Anal.* **25** 340-349.
[27] Naheed Z, Cheng Z, Cuinan W, Yanbin W and Ding H *Postharv. Biol. Technol.* **131** 39-45.
[28] Al-Jadidi H S and Hossain M A 2016 *Asian. Pac. J. Trop. Diseases.* **6** 376-379.
[29] Ruan X, Wen-xia C, Yang L, Zhao-hui L, Ben L and Wang Q 2017 *J. CO_2. Util.* **18** 283-293.
[30] Luyang L, Weifang L, Xiangluan W, Ding Q, Zhang F and Dingrong W 2015 *J. Chromatograph. Sci.* **53** 307-311.
[31] Ajanal M, Gundkalle M B and Nayak S U 2012 *Anc. Sci. Life.* **31** 198-201.
[32] Hunlun C, Beer D, Sigge G O and Jessy V W 2017 *J. Food. Comp. Anal.* **62** 115-125.
[33] Pelitli E P, Janiak M A, Amarowicz R and Alasalvar C 2017 *Food. Chem.* **218** 584-590.
[34] Protti M, Gualandi I, Mandrioli R, Zappoli S, Tonelli D and Mercolini L 2017 *J. Pharma. Biomed. Anal.* https://doi.org/10.1016/j.jpba.2017.05.048.
[35] Leao D P, Franca A S, Oliveira L S, Bastos R and Coimbra M A 2017 *Food. Chem.* **225** 146-153.
[36] Zhang Z, Kong F, Hui N, Zhixian M, Jian-Bo W, Dehong H and Chunyan Y 2016 *Carbohyd. Polym.* **144** 106-114.
[37] Jabbar M and Jabbari A 2106 *Colloid. Surf. A. Physiochem. Engg. Aspect.* **489** 392-399.
[38] Trivittayasil V, Kameya H, Shoji T, Tsuta M, Kokawa M and Sugiyama J 2017 *Food. Chem.* **232** 523-530.
[39] Mirahmadi S F and Norouzi R 2017 *Food. Biosci.* **18** 53-59.
[40] Zhanyuan D and Bramlage W J 1992 *J. Agric. Food. Chem.* **40** 1566-1570.
[41] Alam Z and Fareed U 2016 *J. Anal. Method. Chem.* Article ID 9412767, 1-5.
[42] Ghani M A, Barril C, Bedgood D R and Prenzler P D 2017 *Food. Chem.* **230** 195-207.
[43] Priyanka B, Anitha K, Shirisha K, Janipasha S K, Dipankar B and Rajesh K 2013 *Int. Res. J. Pharm. App. Sci.* **3** 93-101.
[44] Fermando C D and Preethi S 2015 *MethodsX* **2** 283-291.
[45] Madhumitha G and Saral A M 2009 *Asian. J. Chem.* **21** 2468-2470.
[46] Ouerghemmi I, Rebey I B, Rahali F Z, Bourgou S, Pistelli L, Ksouri R, Marzouk B and Tounsi M S 2017 *J. Food. Drug. Anal.* **25** 350-359.
[47] Anupama N and Madhumitha G 2015 *Int. J. PharmTech. Res.* **8** 206-210.
[48] Madhumitha G and Saral A M 2009A *Asian. J. Chem.* **21** 2471-2472.
[49] Yue X F, Shang X, Zhang Z J and Zhang Y N 2017 *J. Food. Drug. Anal.* **25** 327-332.
[50] Semeniuc C A, Pop C R and Rotar A M 2017 *J. Food. Drug. Anal.* **25** 403-408.
[51] Venugopal K, Ahmad H, Manikandam E, Thanigai K, Kavitha K, Moodley M K, Rajagopal K, Balabhaskar R and Bhaskar M 2017 *J. Photochem. Photobiol. B. Biol.* **173** 99-107.
[52] Ranjbarnejad T, Saidijam M, Moradkhani S and Najafi R 2017 *Prost. Other. Lipid. Mediat.* **131** 1-8.
[53] Wang H, Tao L, Tengyang N, Hao G, Feng J, Xiaojun D, Feng J, Ding Y, Weiming X, Shiyu G, Hisamitsu T, Qian Y and Yanqing L 2017 *J. Ethnopharmacol.* **205** 147-157.
[54] Ronald L R, Xianli W and Karen S 2005 *J. Agric. Food. Chem.* **53** 4290-4302.
[55] Karadag A, Ozcelik B and Saner S 2009 *Food. Anal. Method.* **2** 41-60.
[56] Aksoy L, Kolay E, Agilonu Y, Aslan Z and Kargioglu M 2013 *Saudi. J. Biol. Sci.* **20** 235-239.
[57] Nijveldt R J, Nood V, Hoorn D, Boelens P G, Norren K V and Leeuwen V 2001 *Am. J. Clin. Nutr.* **74** 418-425.
[58] Pietta P G 2000 *J. Nat. Prod.* **63** 1035-1042.
[59] Antolovich M, Prenzler P D, Patsalides E, McDonald S and Robards K 2002 *Analyst*. **127** 183-198.
[60] Moein M R, Moein S and Ahmadizadeh S 2008 *Molecul* **13** 2804-2813.
[61] Brewer M S 2011*Comprehen. Rev. Food. Sci. Food. Safe.* **10** 221-247.

[62] Alam N, Brishti N J and Rafiquzzaman M 2013 *Saudi. Pharm. J.* **21** 143-152.
[63] Kariuki D K, Miaron J O, Mugweru J and Kerubo L O 2014 *BEST: Int. J. Human. Art. Med. Sci.* **2** 1-6.
[64] Ibrahim H, Oyi R A, Ehinmidu JO, Musa KY and Bright N Y 2010 *J. Med. Plant. Res.* **4** 1028-1032.
[65] Ahmad N, Rab A and Ahmad N 2016 *J. Photochem. Photobiol B: Biol.* **154** 51-56.
[66] Martins FJ, Caneschi CA, Vieira J L F, Barbos W and Raposo N R B 2016 *J. Photochem. Photobiol B: Biol.* **161** 34-39.
[67] Bazylko A, Borzym J and Parzonko A 2015 *J. Photochem. Photobiol B: Biol.* **149** 189-195.
[68] Andreazza N L, Lourenço C C, Hernandez-Tasco AJ, Pinheiro M L B, Stefanello M E A, Costa E V M and Salvador J 2016 *J. Photochem. Photobiol B: Biol.* **160** 154-162.