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Evaluation of Antioxidant Potential and Radical Scavenging Activity of Organic and Inorganic Fractions of *Cucumis melo*

TANZEEL-UR-REHMAN*, SADAF RIAZ, MUHAMMAD ZAHID QURASHI, PETER JOHN, MUKHTAR-UL-HASSAN and TAHIR RASHEED

Department of Chemistry, Government College University, Lahore-54000, Pakistan

*Corresponding author: Tel: +92 334 4157675; E-mail: tanzeel_u@yahoo.com

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The purpose of this work is to study *in vitro* antioxidant and radical scavenging capacity of *Cucumis melo* components which are effective against different diseases like cardiovascular and kidney disorders. These are the conventionally used herbs against degenerative diseases so it is an important task to assess their antioxidant potential. Extraction of herb was carried out in aqueous as well as in different organic solvents. Antioxidant potential and radical scavenging activity of seven fractions were investigated by using different antioxidant assays like {2,2'-azino-*bis*(3-ethylbenzothiazoline-6-sulphonic acid)} (ABTS), ferric reducing antioxidant power (FRAP), total phenolic contents (TPC) and diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Trolox equivalent antioxidant activity (TEAC) of the aqueous and organic fraction of these herbs was determined by calculating the percentage inhibition of the coloured radical solution after reaction with sample and standard antioxidants by comparing with the standard curve formed by Trolox. *Cucumis melo* showed a wide range of antioxidant activity. Using FRAP assay, TEAC values of from 0.986-1.293 μM and in case of ABTS assay in buffer medium, TEAC values ranged from 0.107-0.691 μM which indicate the presence of many phenolic components in its composition. On the basis of such antidisease activity of this herb attributes the presence of antioxidant components.

Key Words: *Cucumis melo*, 2,2'-Azino-*bis*-(3-ethylbenzothiazoline-6-sulpohonic acid), Trolox equivalent antioxidant capacity.

INTRODUCTION

The free radical reactions generally occur in the mitochondrial respiratory system[1]. Free radicals or oxidative injury now appears the fundamental mechanism underlying a number of human neurologic and other disorders[2]. For instance, in diabetes, increased oxidative imbalance which co-exists with reduction in the antioxidant status has been postulated[3]. Presently, the possible toxicity of synthetic antioxidants has been evaluated. Generally it is assumed that frequent utilization of plant-derived phytochemicals from vegetables, fruit and herbs may contribute to shift the balance toward an adequate antioxidant status. Such interest in natural antioxidant, has frequently increased in recent years[4].

There are many classes of antioxidant dietary compounds have been recommended to present health benefits and there are evidences that utilization of these products leads to a reduction of the expression of a variety of pro-inflammatory and/or oxidative stress biomarkers[5-7]. The active principles in these vegetal extracts are principally water soluble or lipophilic antioxidant molecules. Infact, most of these plant extracts contain diverse amounts of vitamin E, vitamin C, carotene and other flavonoids[8,9] and were used as potential antioxidant prophylactic agents for both health and disease managing[6,10-13]. However and until now it was not possible to use the antioxidant enzymes [*e.g.*, superoxide dismutase (SOD) *etc.*] naturally present in various plant extracts[14,15] as nutritional supplement. Indeed, these antioxidant enzymes are usually inactivated and digested all along the gastro-intestinal transit thus destroying the antioxidant pharmacological properties of these detoxifying proteins[16,17].

Cucumis melo is generally called as Muskmelon. A previous study showed that cantaloupe pulp extract possesses high antioxidant and antiinflammatory properties[18]. However, antioxidant assessment on different parts of *Cucumis melo* is very limited[19].

Although some preliminary studies on antioxidative potential of the plant extracts and its herbomineral formulations have been carried out[20,21] but no comprehensive study on the radical scavenging and antioxidant capacity has been undertaken as yet. The objective of the present study is to evaluate radical scavenging and antioxidant potential of different organic and aqueous extracts of *Cucumis melo* by using trolox equivalent antioxidant capacity (TEAC) assay, ferric reducing antioxidant

power (FRAP) assay, total phenolic contents (TPC) assay, DPPH free radical scavenging assay and ABTS decolourization essay.

EXPERIMENTAL

Cucumis melo was purchased from a local market, Paapar Mandi, Lahore, in January 2010 and identified by Muhammad Ajaib (Taxonomist), Department of Botany, Government College University, Lahore.

Extraction of antioxidant components: Socked 10 g of finely ground herb in 100 % methanol (4 × 200 mL) at room temperature with mild shaking for 48 h. The extract was filtered out and the residue was extracted again to ensure complete extraction. From 100 % of the filtrate, methanol was evaporated under reduced pressure to obtain a crude residue. The residue was re-suspended in distilled water (200 mL). The aqueous solution was successively partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol (4 × 25 mL for each extraction). The non-aqueous layer was separated and stored at 4 ºC until used for further analysis.

Chemicals and standards: Standard antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxlic acid) and other chemicals such as 2,2,-diphenyl-1-picrylhydrazine (DPPH) ICN biomedical I, *n*-hexane, acetone, methanol, acetic acid, ethyl acetate, ethyl alcohol, ferric chloride, sodium chloride, sodium acetate, dichloromethane, potassium persulfate, dipotassium hydrogen phosphate, potassium dihydrogen phosphate, all of them are E. Merck, deionized water, 2,2-azino-*bis*-3-ethyl benzothiazoline-6-sulphonic acid (ABTS), I-diammonium salt, Aldrich chemical Co., 2,4,6-tripyridal-s-triazine (TPTZ), HCl. Follin-Ciocalteau's reagent and potassium persulfate (dipotassium peroxdisulfate) were purchased from Fluka (UK). HPLC grade ethanol was purchased from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire, Scotland). Spectrophotometric measurements were made on UV-1700 PharmaSpec. UV-Visible spectrophotometer, Shimadzu, Japan equipped with temperature control device. All the solutions were made in triplicate and experiments were performed three times. The results obtained were averaged.

$ABTS^{\bullet+}$ radical cation decolourization assay: (In buffer media): "ABTS" radical scavenging assay depends upon the scavenging activity of ABTS radical cation generated from a reaction between ABTS and 3.49 mL of (10 mM) potassium persulfate (K_2SO_4) and making the total volume to 14.28 mL by adding 0.780 mL of deionized water in it. The antioxidant components changes bluish green ABTS radical to colourless native ABTS depending upon their nature and quantity on a time-dependant scale. Trolox is usually used as a standard antioxidant for assessment purposes. For the determination of TEAC value of the extract decolourization assay was followed[22]. ABTS radical cation was produced by a reaction between ABTS and potassium persulfate (7 and 2.45 mM final concentrations, respectively) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use and intense bluish green coloured stable radical cation ($ABTS^{+}$) was generated. To study the antioxidant activity of standard antioxidant and indigenous medicinal herb, the ABTS stock solution was diluted with PBS buffer (pH 7.4) to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 ºC. Then 3.49 mL of diluted $ABTS^{\bullet+}$ solution (A_{734} nm = 0.70 + 0.020) was transferred into the cuvette and noted down the absorbance as A_o. Then added 10 µL of sample solution, the absorbance reading was taken as 25 ºC exactly 1 min after initial mixing and upto 6 min. Appropriate blank were run in each case. All determinations were carried out at least three times in succession and in triplicate at each separate concentration level of the standards. The percentage inhibition of absorbance was calculated by the following formula.

$$\text{Inhibition } (\%)\,(\text{at } 734 \text{ nm}) = \left(1 - \frac{I_f}{I_o}\right) \times 100$$

where I_o = absorbance of radical cation solution before addition of sample/standard antioxidants and I_f = absorbance after addition of the sample/standard antioxidants. $ABTS^{\bullet+}$ radical cation also prepared by using methanol. The resultant data was plotted between concentration of antioxidants and that of Trolox for the standard reference curve.

Total phenolic contents assay (TPC): Total phenolic contents of the extracts were determined by a reported method[23]. Stock solution of gallic acid was made by dissolving 0.500 g gallic acid in 10 mL of C_2H_5OH in a 100 mL conical flask and diluted it to volume with double distilled water. Sodium carbonate solution was prepared by dissolving 200 g of anhydrous Na_2CO_3 in 800 mL of distilled water. After boiling and subsequent cooling of the solution, a few crystals of sodium carbonate were added. The solution was stand for 24 h, filtered and volume was raised to 1 L with distilled water. To prepare a calibration curve, 0, 1, 2, 3, 5 and 10 mL of stock solution of phenol were added into 100 mL conical flask separately and then diluted to volume with distilled water. The final solutions contained concentrations of 0, 50, 100, 150, 250 and 500 mg/L gallic acid, the effective range of assay. From each calibration solution and sample or blank, 40 µL were pipetted into separate cuvettes and to each 3.16 mL of distilled water was added. Folin-Ciocalteu's reagent (200 µL) was added and mixed well. After 8 min, 600 µL of Na_2CO_3 solution was mixed thoroughly in the solution. The solution was allowed to stand at 20 ºC for 2 h and absorbance of each solution was noted at 765 nm against the blank. A concentration *versus* absorbance linear plot was thus obtained. Alternately, they can be left at 40 ºC for 0.5 h before reading the absorbance and noted the absorbance at 765 nm. Create a calibration curve with standard and determine the level in sample.

Ferric ion reducing antioxidant power assay (FRAP): The ferric ion reducing capacity of plant extract was measured according to the reported method[24]. Freshly prepared FRAP solution contained 25 mL of 300 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid solution and 2.5 mL of 20 mM ferric chloride ($FeCl_3$) solution. The mixture was incubated at 37 ºC throughout the reaction period. 3 mL of FRAP reagent was mixed with 100 µL of sample and 300 µL of distilled water. Absorbance readings were taken at 593 nm after every minute for 6 min. Results were compared with standard curve of ferrous sulphate.

2,2'-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay (DPPH): 2,2'-Diphenyl-1-picrylhydrazyl free

radical scavenging potential was found by using a previously reported method[25]. 2,2'-Diphenyl-1-picrylhydrazyl is one of a few stable and commercially available organic nitrogen radical and has a UV-vis absorption maximum at 515 nm. Upon reaction, solution colour fades and the reaction progress is monitored by a spectrophotometer.

Briefly, DPPH solution (3 mL, 25 mg/L) in methanol was mixed with appropriate volumes of neat or diluted sample solutions (0.1 mL). The reaction progress of the mixture was monitored at 517 nm over a time period of 0.5 h until the absorbance becomes stable. Upon appropriate reduction, the purple colour of the solution changed to yellow diphenyl-picrylhydrazine. The percentage of the DPPH remaining ($DPPH_{rem}$ %) was calculated as

$$DPPH_{rem}\ (\%) = 100 \times \frac{[DPPH]_{t=t}}{[DPPH]_{t=0}}$$

where $[DPPH]_{t=0}$ = concentration of DPPH radical before reaction with antioxidant samples and while $DPPH_{rem}$ = proportional to the antioxidant concentration and $[DPPH]_{t=t}$ = concentration of DPPH radical after reaction with antioxidant sample at time t. A kinetic curve showing the scavenging of DPPH radical in terms of decrease in absorbance at 517 nm as a function of time (min) was plotted for each fraction of the samples. EC_{50} value, which is the concentration of a substance that reduces the amount of DPPH radical to half of the original concentration under experimental conditions, was also determined for each fraction.

RESULTS AND DISCUSSION

$ABTS^{\bullet+}$ decolourization assay: The ABTS decolourization assay was applied to evaluate *in vitro* radical scavenging potential of different fractions of *Cucumis melo*. The reduction potential of the ABTS radical cation is comparable to that of hydroxyl radical produced during metabolic reactions *in vivo*. Trolox equivalent antioxidant capacity (TEAC) values were obtained by comparing the percentage inhibition values of samples (solvent fractions) with the standard trolox curve. Column graphs are plotted for the TEAC values of each fraction of the sample (Fig. 1). Trolox equivalent antioxidant capacity values in buffer medium ranged from 0.107-0.691 µM of Trolox equivalents. Amongst different fractions, aqueous extract before partitioning, aqueous extract after partitioning and 1-butanol fractions showed higher TEAC values than those ethyl acetate, *n*-hexane, chloroform and methanol. Two media were used for the generation of ABTS free radical, one was methanloic and other was buffer media, but buffer media shows more percentage inhibition. In methanolic medium maximum peak value (0.186) is given by aqueous extract before partitioning, which indicates that it has maximum radical scavenging activity and minimum in methanol. In buffer medium more radical scavenging capacities as compared to methanolic medium. Order of radical scavenging activity of different fractions in buffer medium as aq. (AP) > aq. (BP) > 1-butanol > ethyl acetate > *n*-hexane > chloroform > metahnol (Fig. 1A-C).

Total phenolic contents: Folin-Ciocalteu reagent is usually used in the laboratories for the determination of phenolic components in plants/herbal extracts and other fractions. Phenolic

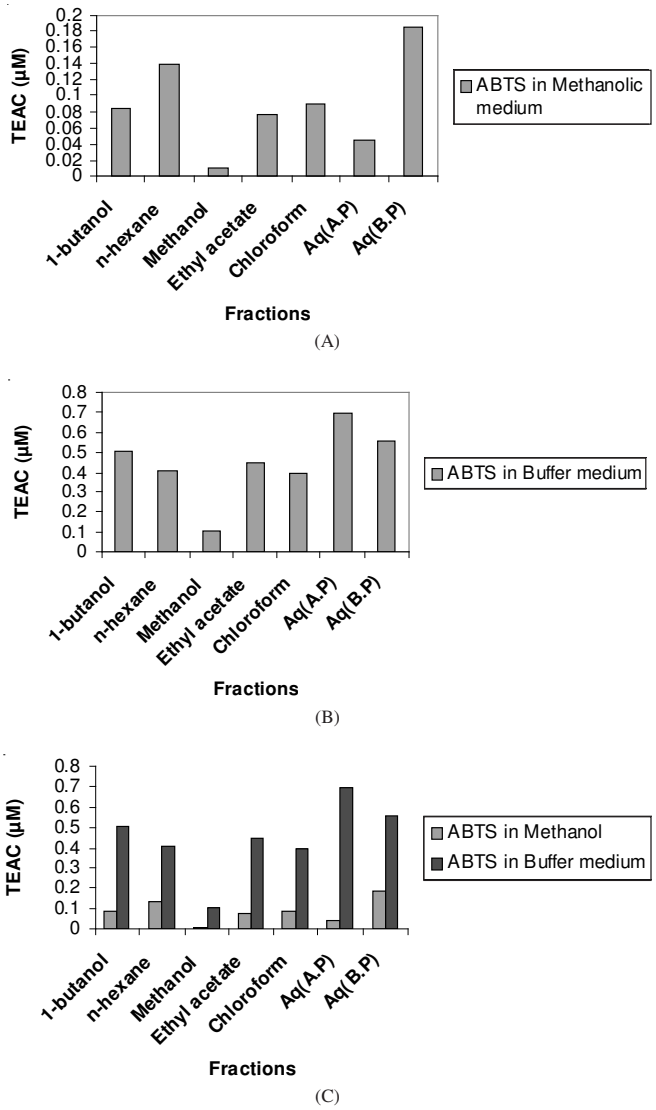

Fig. 1. Trolox equivalent antioxidant capacity values of extracts of *Cucumis melo* by ABTS assay (A) in methanolic medium (B) in buffer medium, (C) comparison between methanolic and buffer medium

compounds react Folin-Ciocalteu reagent only under basic conditions at pH 10. Hydroxyl moieties of phenolic compounds have the ability to reduce yellow coloured Folin-Ciocalteu reagent to blue colour. The change in the colour is monitored spectrophotometrically at 765 nm. Total phenolic content values ranged from 0.171-0.517 µM of GAE/100 g of dry weight (Fig. 2). Employing total phenolic content assay, the order of antioxidant activity of different fractions of *Cucumis melo* was found to be ethyl acetate > aq. extract (before partitioning) = methanol > 1-butanol > aq. extract (after partitioning) > chloroform > *n*-hexane.

Ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power assay is employed to assess antioxidant power by knowing the ability of the sample to reduce ferric ion to ferrous ion at low pH. The ferric ion reducing antioxidant power assay as developed by Benzie and Strain which involves a single electron reduction of the $Fe(TPTZ)_2(III)$ complex (pale yellow) to the $Fe(TPTZ)_2(II)$ complex (blue) by sample antioxidants at acidic pH. Any antioxidant species with lower reduction potential than that of Fe(III)TPTZ

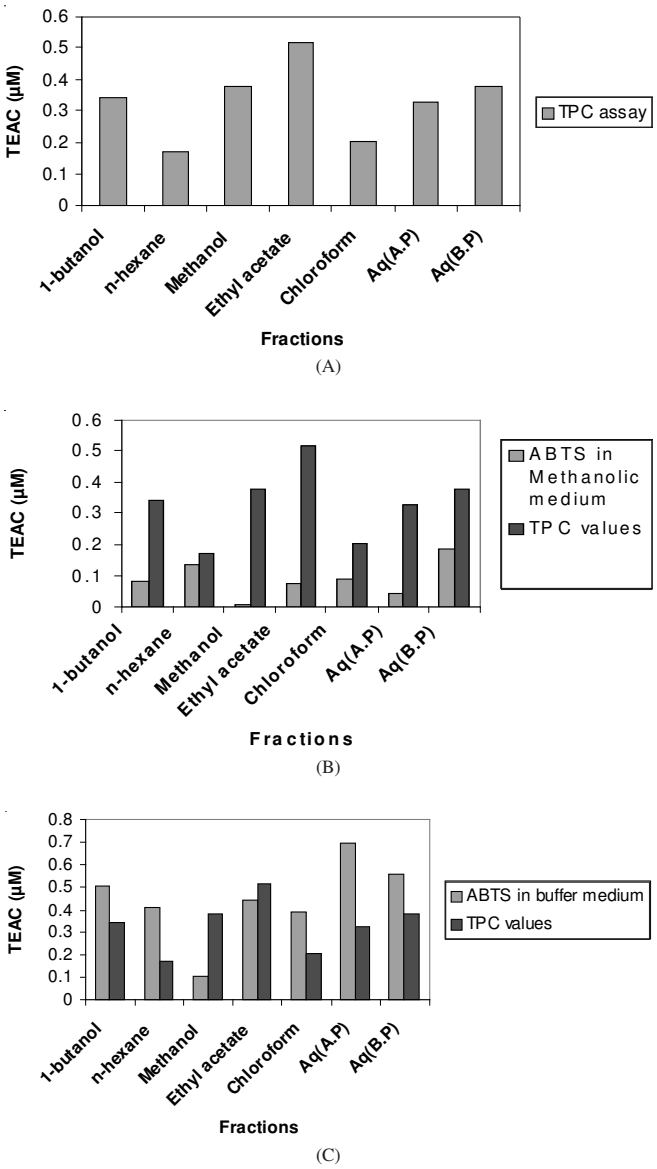

Fig. 2. (A) Trolox equivalent antioxidant capacity values of *Cucumis melo* by using total phenolic contents assay (B) comparison between TEAC values of ABTS in methanilc medium and TPC values (C) comparison between TEAC values of ABTS in buffer medium and TPC values

salt (0.7 V) can reduce Fe^{3+}-TPTZ to Fe^{2+}-TPTZ contributing to ferric reducing antioxidant power value[26]. This reduction is progressed spectrophotometrically at 593 nm. Appearance of intense blue colouration show the presence of reducing components in the sample. The original method of Benzie and Strain uses a 4 min interval but it is noted that the reaction/colour change is in progress even after 4 min interval. Absorbance readings, therefore, were taken at a 6 min interval after addition of sample to TPTZ reagent allowing the reaction to reach a steady state. Ferric reducing antioxidant power values for different fraction ranged from 1.293-0.986 µM (Fig. 3). Higher TEAC values for methanol, *n*-hexane, 1-butanol and aqueous extract (before partitioning).

2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH): DPPH is preformed to measure radical scavenging activity of antioxidant samples and has a UV visible absorption maximum at 515 nm. Upon reaction the solution colour fades.

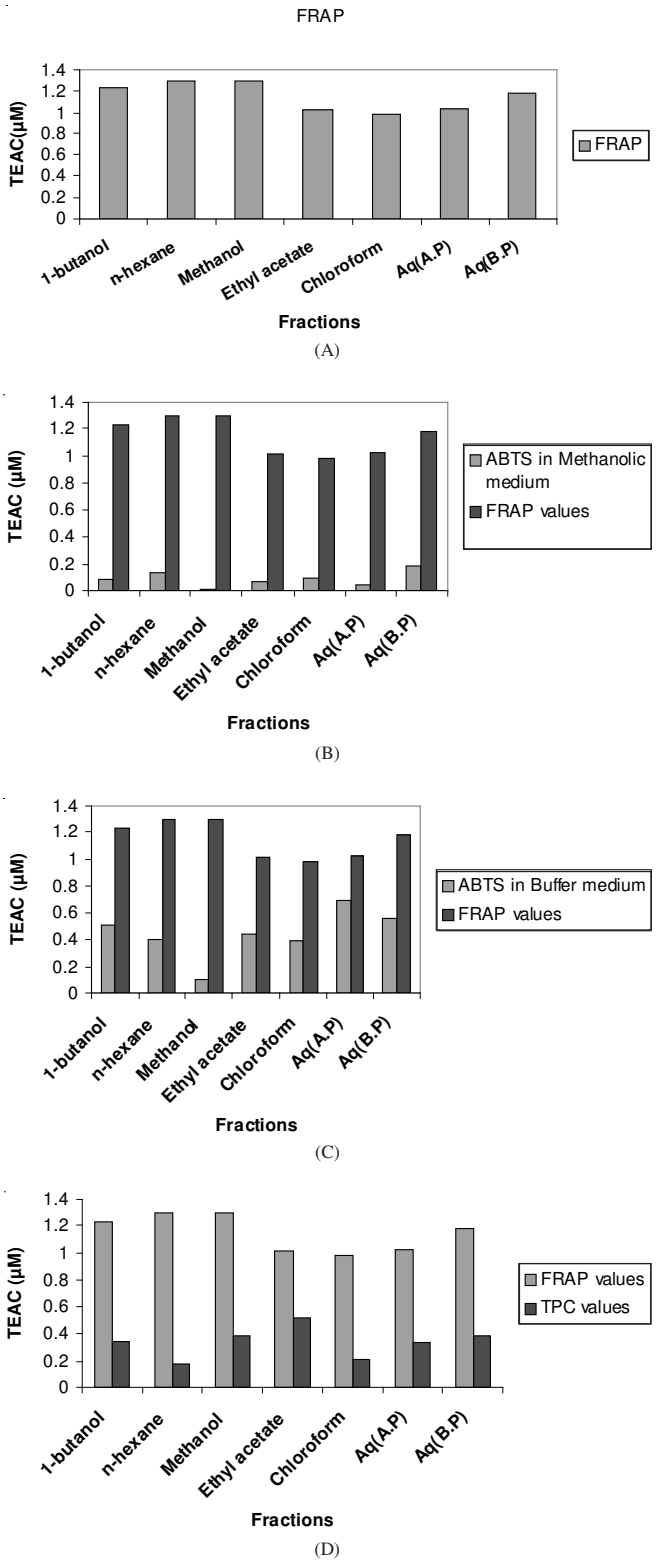

Fig. 3. (A) Trolox equivalent antioxidant capacity values of the fractions of *Cucumis melo* by FRAP assay (B) comparison between TEAC values of ABTS in methanolic medium and FRAP values (C) comparison between TEAC values of ABTS in buffer medium and FRAP values (D) Comparison between FRAP values and TPC values

The progress of the reaction is monitored by a spectrophotometer. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay was performed by taking parameters, the absorbance of antioxidant and the time for completion of the reaction of antioxidants, into consideration. Kinetic curves obtained by

plotting absorbance against time showed that all the fractions of *Cucumis melo* contained high levels of DPPH radical scavenging agents (Fig. 4). It is clear from the figure that the curves obtained from ethyl acetate is much steeper in the first 15 min, showing fast reaction of antioxidant components with DPPH radical and all other fractions show the curve almost parallel at its origin, indicates the completion of the antioxidants.

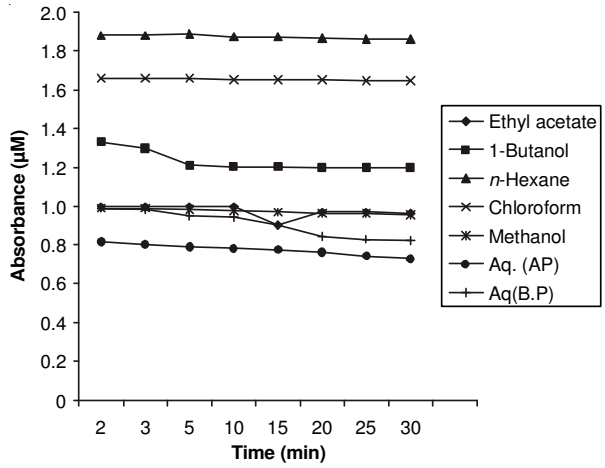

Fig. 4. Absorbance values of different fractions of *C. melo* by DPPH assay

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