Determination of Total phenolic content, Antioxidant capacity and radical scavenging capacity of Vegetable Biomass

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***Abstract*— Vegetable biomass is the source taken for the extraction of antioxidants. The main goal of the present work is to determine the optimal conditions for the extraction of antioxidant compounds from the vegetable biomass using aqueous solutions of methanol and to investigate the total phenols content, total antioxidant capacity and the radical scavenging activity of extracts made. The maximum yield was determined for the extraction carried out under different parameters. At first, several preliminary tests were conducted to study extraction process under selected conditions (50% aqueous solution of methanol at different temperatures and time).The maximum values showed are, Total phenolic content: 0.04 mg/g GAE, Ferric reducing capacity: 3.729µm Fe(II)/gm of extractand the percent of radical scavenge is 70.38(%).**

***Index Terms*— Vegetable biomass, Total phenolic content, F-C reagent, and Total antioxidant capacity.**

I. INTRODUCTION

Antioxidants is the molecules that inhibits the oxidation of other molecules. Antioxidants terminates the chain reaction by removing free radical termination intermediates in inhibits other oxidation reactions. They do this by oxidising themselves so antioxidants are often reducing agents. Such as, thiols, ascorbic acid or polyphenols. Antioxidants are of two forms, Natural antioxidants and artificial antioxidants. Natural antioxidants are obtained from the day today dietary supplements. These natural antioxidants are present plenty in natural green plants and vegetables. Artificial antioxidants are synthetically made by the chemical substances. The natural antioxidants are always good to consume in nature. Fresh fruits and vegetables contains ascorbic acid as an antioxidants components, vegetable oils consists of tocopherols, tocotrienols as antioxidants components. Tea, coffee, soy fruit, olive oil, chocolate, cinnamon, oregano consists of resveratrol, flavonoids and antioxidants components. Fruits, vegetables and eggs consists of lycopenes, carotene. On the other hand, many research prove that artificial antioxidants such as, Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), are harmful to the lab animals and they cause disease that lead to liver damage cytotoxicity and carcinogenesis [1]. Thus to the side on the healthier of life the natural antioxidants is always preferable to health. The work has been aimed to bring out the antioxidant property and radical scavenging capacity of the vegetable biomass, which is being obtained intones from local markets. The vegetable biomass, consists of vegetable that are normally available in the markets of South India which is undergone some research for the presence of polyphenols components with antioxidant activity[2-38]. These vegetable biomass is made to dry, then extracted with methanol by varying parameters (time, temperature and concentration) and extract are made to undergo analysis for total phenols contents by FolinCiocalteu, ferric reducing antioxidant capacity by Oyaizu (1986) method and Hydrogen peroxide radical scavenging capacity.

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II. MATERIALS AND METHODS

A. *Chemicals*

Gallic acid (CAS number 497-19-8), sodium carbonate (CAS number 497-19-8), FolinCiocalteu reagent,L-ascorbic acid (CAS number 50-81-7), iron (III) chloride hexahydrate (CAS number 10025-77-1), acetic acid(CAS number 64-19-7), Methanol (CAS number 67-56-1), Potassium ferric cyanide (CAS13746-66-2), Di Sodium hydrogen phosphate (CAS 7558-79-4), Sodium di hydrogen Phosphate (CAS 89140-32-9),Trichloro acetic acid (CAS76-03-9),Hydrogen peroxide (CAS7722-84-1).

B. *Collection of vegetable biomass*

The vegetable biomass was at wholesale vegetable complex(13°04'00.9"N, 80°11'47.8"E), koyambedu, Chennai.

C. *Preparation of vegetable biomass*

a) *Cleaning:*

The vegetable biomass collected is cleaned well with distilled water for the removal of dirt and other unwanted materials.

b) Drying:

The well cleaned bio mass is cut to small pieces for quick drying and subjected to dying in shade for about 45°C

D. Extraction procedure

Extraction of antioxidant compounds from vegetable biomass was carried out with methanol-water solutions in an orbital shaker. The solid - liquid ratio was set at 1:10 (w/v) for all experiments. Temperature (°C), time (minutes) and solvent concentration (% volume fraction) varied depending on the experimental stage.In a first stage, experiments were performed toanalyse the influence of extraction time (from 10 to 30 min) and temperature (30, 40, 50 and 55˚C) on the antioxidant extraction process. Methanol concentration was kept constant at 50% (volume fraction), the central point of the range analysed in the next stage (20 - 90%).The methanolic extract obtained from the extraction of dried vegetable biomass with methanol was vacuum evaporated for the recovery of methanol from the extract- methanol mixture. The methanol free extract was vacuum dried at temperature of 30˚C at constant pressure of 400mm Hg.Theextraction yield was calculated using the formula,

$$\% \text{ Yield} = \frac{\text{Weight of dried sample x 100}}{\text{Weight of original sample}}$$

Sample no.	Concentration % volumefraction)	Time (mins)	Temp (˚C)
1.	20	10	30
2.	60	30	55
3.	90	10	40
4.	60	10	50
5.	20	10	50
6.	90	10	30

Table 3.1: Extraction Parameters

III. DETERMINATION OF TOTALPHENOLICCONTENTS

The amount of total phenolicinextracts was determined according to the Fol in- Ciocalteu method[39].Gallic acid was used a sastandard and the total phenolic were expressed asmg/g gallicacid equivalents (GAE).Adilute extract of each plant extract(0.5mlof1:5g/ml) orGallic acid isused as standard was mixed with Folin-Ciocalteaureagent(2.5ml,1:10dilutedwithdistilledwater) and aqueous 7.5% ofNa_2CO_3(2ml,1M). The mixture was allowedtostandfor10minandabsorbancewasmeasuredbycalorimetricallyat760nmthestandardcurve was prepared using 0.002,0.004,0.006,0.008,0.010,0.012 mg/mlof solutions of gallicacid in methanol- water solutions. All determination was performed in triplicate. The Folin-Ciocalteureagentis sensitive to reducing compounds including polyphenols, thereby producing a blue colour chromogens upon reaction. This blue colour ismeasured spectrophotometrically. Thus total phenolic content can be determinedby the formula,C = c.V/M, Where, C – total phenolic content (mg/gGAE), c – Concentration of gallicacid (mg/ml), V- volume of extract in assay (ml), M – Mass of pure plant methanolic extract (gm).

IV. REDUCING POWER CAPACITY (OYAIZU- 1986)

The reducing power capacity is experimentally by the method of Oyaizu (1986) as described by V.Y.A Barku et al[40].The extract is diluted with methanol at 1ml: 5ml. From the above different concentrations (1, 3, 5, 7,9,11 g/ml) were pipetted out. 2.5ml of 1% potassium ferric cyanide and 2.5ml of 0.2M sodium phosphate buffer (pH 6.6) briefly, preparing 3.5% disodium phosphate(A) and 3.1% sodium di hydrogen phosphate(B). From pipetting out 26.5 ml of solution A and 73.5 ml of solution B and makingup it to 200 ml and adjust pH using pH meter. This gives phosphate buffer of 0.2M.The mixture will be incubated at 50°C for 20 minutes, and the reaction is terminated by the addition of 2.5 ml of 10% (w/v) trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 minutes. 2.5ml of the supernatant upper layer will be mixed with 2.5 ml distilled water and 0.5ml of 0.1% iron (III) chloride hexahydrate, which turns the reaction mixture for extract to green colour and absorbance will be measured at wavelength of 700nm.This method is based on principle that the increased absorbance indicated increase in reducing power of the sample, thus the antioxidant values are obtained.All determinationwasperformedintriplicate. The control contained all the reagents except the sample. Ascorbic acid is used as positive control.

V. HYDROGEN PEROXIDE SCAVENGING CAPACITY

The scavenging activity of extract towards hydrogenperoxideradicals was determined by the modified method of Dehpour as discussed in Frank Ngonda [37].Solution of hydro genperoxide(40Mm) was prepare dinphosphatebuffer(pH7.4) andits concentration was determined by measuring the absorbance at 560nm using UVspectrophotometer.0.1mg/ml of the extract was addedtohydrogenperoxide solution and absorbance measuredat560nmusingUVspectrophotometeragainst a blank solution containingphosphate buffer without hydrogenperoxide. The experiment was repeate dintriplicate.The percentage of hydrogenperoxide scavengingby the extract and standard compound was calculatedusing the given formula:Percentage$scavenged = 1 - A_s/A_c x100$, Where, $A_c$was the absorbance of the control (without extract)at560nm;A_s was the absorbance in the presence of the extract at 560nm.

VI. RESULTS AND DISCUSSION

A. Total Phenolics Content

The experiment for the determination of the total Phenolics content was done by the method discussed earlier, the standard curve of the standard compound (Gallic acid) is given in fig. 4.1

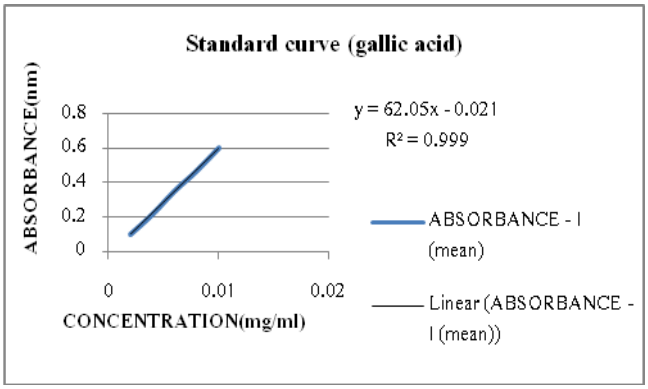

Fig 4.1:Standard curve of Gallic acid

The standard absorbance curve for the extract is obtained by the determination of calculating the concentration from equation from the standard curve, y = 62.05x-0.0211 where Regression value is (R^{2} = 0.9995).The curve obtained for the absorbance of the extract gives the equation of y = 0.0051x + 0.1241 and has the regression value of R^2 = 0.92

Sample No.	Total phenolic content(mg/gGAE)
1.	0.00668±0.006
2.	0.01066±0.002
3.	0.00025±0.005
4.	0.01198±0.001
5.	0.04±0.001
6.	0.01331±0.002

Table4.1**:** Total Phenolic content in Vegetable Biomass extract

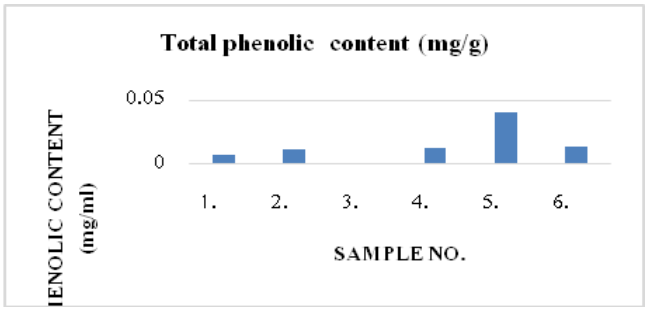

Fig 4.2: Total Phenolic content in Vegetable biomass extract in mg/g (GAE)

Thus the peak in fig 4.2 shows that the Total Phenolic content evaluated from sample no. 5 gives the maximum value of 0.04mg/g GAE.

B. Reducing power capacity

The experiment for the determination of the Reducing power capacity was done by the method discussedand the absorbance values and standard curve of the standard compound (ascorbic acid) and the extract for the wavelength 700 nm is given in fig4.3.At the wavelength of 700 nm, the 3rd sample showed the maximum absorbance of 3.879 µm Fe (II)/gm of extract.

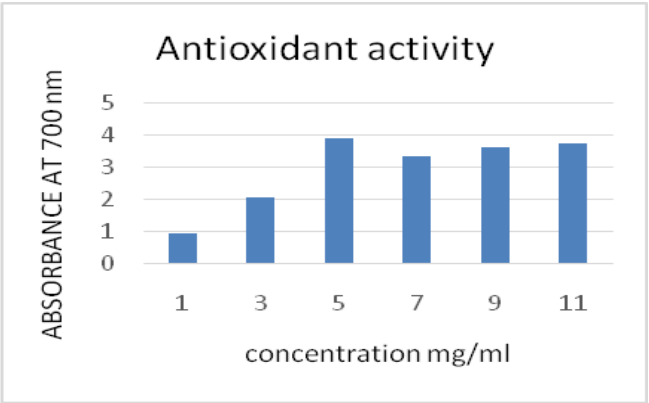

Fig 4.3: Antioxidant Capacity

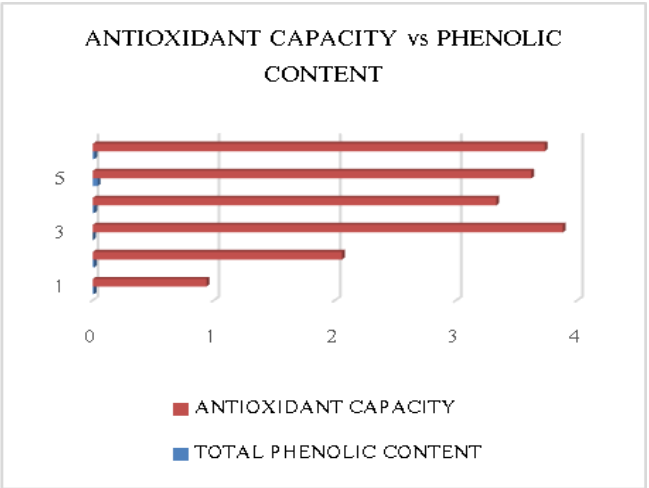

Fig 4.4: Total Phenolic Content vs. Antioxidant Capacity

The comparison in fig 4.4 from the data obtained shows that though the phenolic content values are less in the extracts when compared to antioxidant capacity of the samples extracted with same extraction parameters.

C. Hydrogen peroxide scavenging capacity

The % Radical Scavenged values calculated from the formula discussed in table 4.3.The 4th sample showed maximum percentage Radical Scavenged of 70.38 (%) in extracts.

Sample No.	% Radical Scavenged (mean)
1	66.97
2	64.6
3	58.6
4	70.38
5	47.05
6	24.07

Table 4.3: % Radical Scavenged values

VII. CONCLUSION

Based upon the results obtained from the current study, it is concluded that the methanolic extract of Vegetable biomass of 10g contains total phenolic content of 5th sample (20% methanolic conc,10 mins,50°c) is 0.04;Total flavonoids content 5th sample (20% methanolic conc,10 mins,50°c) is 94.25mg/g ;Ferric reducing capacity of 3rd sample is (90% methanolic concentration,10 mins and 40°C) ;Hydrogen

peroxide value of 6th sample (90% methanolic concentration,10 mins and 30°C) . It also chelates iron and has reducing power. These indicate that the extract of vegetable biomass is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stress and treating cancer. However, further isolation of bioactive compounds would assist to ascertain its potency and safety as a lead candidate of antioxidant for pharmaceutical uses.

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