Estimation of Total Phenolics and Antioxidant Activity of an Ayurvedic Formulation Containing Aerial Root of F.Bengalensis

Sugandha S. Shetye[a] ,Valsamma Wilson[b], ,and Kawaljit Kaur[c]
a. Department of Chemistry, K. J. Somaiya College of Science and Commerce, Vidhyavihar, Mumbai-77. India.
b. Department of Chemistry, V.K.Krishna Menon College of Commerce and Economics &S.S.Dighe College of Science, Bhandup(E), Mumbai-42. India.
c.Department of Chemistry, ICLES' MJ College of Arts, Science and Commerce, Plot No.53, Sector-9/A, Vashi, Navi Mumbai-400 703. India.

Abstract

A traditional Ayurvedic formulation 'Vizhal Veradi Kasayam' containing the aerial root of F. bengalensis as the major constituent was analyzed for reducing power ability , antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and for total Phenolics using the Folin-Ciocalteu method. The total Phenolics determination showed that the formulation has a Phenolics content of 5.786 mg/mL GAE. Linearity range was found to be 20-140 µg/mL with the correlation coefficient of 0.999.The limit of detection and limit of quantification values were found to be1.55µg/mL and 4.65 µg/mL .The formulation showed significant antiradical activity by bleaching 1,1-diphenyl-2-picrylhydrazyl radical (IC_{50} 8.5µg/mL) which is much better than that of Ascorbic acid (IC_{50} 22.0µg/mL). In this simple spectrophotometric method aqueous and alcoholic extracts were used for analysis. The reducing ability, Fe^{3+} to Fe^{2+}transformation was found to increase with increasing concentrations of the sample.

KEYWORDS:-DPPH assay, F. bengalensis. Total Phenolics, Antioxidant activity.

INTRODUCTION

In the living system normally free radicals of different forms are generated in cells to help in the modulation of several physiological functions. A certain level of free radicals is essential for good health as they are involved in fighting infection and in the contraction of smooth muscles in the blood vessels. Cells have a number of ways of dealing with excess free radicals including the use of enzyme systems and antioxidants(Mattil 1947)(W. 2002) Endogenous and exogenous antioxidants acts as free radical scavengers by preventing and repairing damages caused by the reactive oxygen species and enhance the immune defense and lower the risk of cancer and degenerative diseases [(Valko m 2005),(Valko M 2006),(Parthasarathy S 1999),(Chatterjee m 2007)].Antioxidants neutralize free radicals before they attack healthy cells(Davies 1995). However, if produced in excess amount they can be destructive leading to inflammation, ischemia, lung damage and other degenerative diseases (Halliwell B 1992). The phenolic compounds such as Phenolics acids, biphenyls, flavonoids, and polyphenols like proanthrocyanidins, tannins, have been studied for their antioxidant/free radical scavenging properties[(Larson 1998), ((Harbone 1980), InBella EA 1980),(Haslam 1998)].Antioxidants prevent the damage done to cells by free radicals-

molecules that are released during the normal metabolic process of oxidation. Studies around the world have identified many constituents with antioxidant activity, among these are the polyphenols(Kahkonen MP 1999). The antioxidant activity of polyphenols has been reported to be mainly due to their redox properties, which can play an important role in neutralizing free radical and quenching oxygen or decomposing peroxides. Polyphenols of plant origin like catechins exert anticarcinogenic, antimutagenic and cardio protective effects, which is attributed to their free radical scavenging activity(JD.Habila 2010).

The bark ,leaves and fruits of Ficus species are used as astringent, haemostatic, anti-septic, anti-inflammatory, antioxidant , anticancer agent .They are also used in the treatment of diarrhea, dysentery, skin diseases, ulcers, vaginal disorders, leucorrhoea, menorrhagia, and deficient lactation(Nadkarni 1954). F.bengalensis is an important constituent of 'Vizhal veradi kashayam' a traditional Ayurvedic formulation. This formulation is prescribed by medical practitioners for the treatment of pain and inflammation of arthritis affected joints, in–combination with other formulations. The present study aims at the quantitative estimation of the free radical scavenging ability reducing power and the total phenolics of 'vizhal verady kashayam'.

MATERIALS AND METHODOLOGY:-

Three different batches Vizhal veradi kashayam was purchased from Kottackal Aryavaidyasala. and analysed. Folin Ceocalteu's reagent , Potassium ferricyanide, Trichloro acetic acid, Ferric chloride and Ascorbic acid were purchased from S. D.fine chemicals, Mumbai, India. Gallic acid and 1,1-Diphenyl-2-picryl hydrazil (DPPH) were purchased from Sigma Aldrich. U V Visible Spectrophotometer (Elico-India. SL-207 Equiptronics-EQ820) was used for analysis.

The Phenolics content present was estimated according to the method described by Singleton and Rossi (Singleton VL 1965)using Folin Ceocalteu reagent. The free radical scavenging activity of the formulation was tested using DPPH and reducing power assay by the transformation of Fe^{3+} to Fe^{2+}.

Estimation of Total Phenolics Content:

The total Phenolics content of the formulation was estimated according to the method described by Singleton and Rossi(Singleton VL 1965). 10mg of Gallic acid was dissolved in 100 ml distilled water in a volumetric flask (100 µg/ml of stock solution). From the above stock solution 0.2 to 1.4 ml of aliquots were pipette out into 25 ml volumetric flasks. 10 ml of distilled water and 1.5 ml of Folin Ciocalteu's reagent (diluted according to the label specification) were added to each of the above volumetric flasks. After 5 min, 4 ml of 20% sodium carbonate solution was added. The volume was made up to 25 ml with distilled water and incubated at room temperature for 30 min. The absorbance of the solution was recorded at 685 nm and a standard curve of absorbance verses concentration of Gallic acid (20-140 µg) was plotted.

Aliquots of 50ppm sample solution were taken into a 25 ml volumetric flask. 10 ml of water and 1.5 ml of Folin Ciocalteu's reagent were added to it. The mixture was kept for

5 min. Then 4 ml of 20% sodium carbonate solution was added and made up to 25 ml with double distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was recorded at 685 nm. Percentage of total Phenolics was expressed in terms of Gallic acid as Gallic acid Equivalents (GAE).

Reducing Power Assay:

The reducing power of the formulation was measured by the transformation of Fe^{3+} to Fe^{2+}. Increased absorbance of the reaction mixture indicates increased reducing power (Ravishankara MN 2002). Aliquots of the formulation were mixed with 2.5 ml of phosphate buffer and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 50° for 20 min, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, centrifuged at 3000 rpm for 10 min. 2.5 ml of upper layer of the mixture was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% $FeCl_3$ solution. The absorbance was measured at 700 nm. Gallic Acid was used as positive control.

Free Radical Scavenging Activity:

Free radical scavenging activity was measured by a decrease in absorbance at 517 nm of a solution of coloured DPPH in methanol brought about by the sample (Vani T 1997). A stock solution of DPPH (0.2milli molar in methanol) was prepared such that 2.0 mL of it gave an initial absorbance of 0.98. Decrease in the absorbance by the different concentrations of sample was noted after 15 min. IC_{50} was calculated from percentage antioxidant activity. Methanol was used as blank and Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION;-

Total Phenolics:

The total Phenolics present in the formulation were estimated as Gallic acid equivalent using the calibration curve plotted with absorbance against concentration of Gallic acid. It is estimated as 5.786±0.022mg/mL

Linearity and Range

Linearity was studied by preparing standard solution at different concentration levels. The linearity range was found to be 20-140 µg/mL of Gallic acid. The regression equation was found to be y=0.003x+0.002 with coefficient of correlation, r2=0.999.where x is concentration; y is absorbance.0.00333 is the slope and 0.002 is the intercept .Percentage curve fitting was found to be 99.9%. [Table 1], [Fig.1].

Limit of Detection and quantification;-

Every analytical method has a detection limit. For methods that employ a calibration curve it is calculated from the slope of the calibration curve and the standard deviation of the blank. Here it was found to be 1.55µg/mL and quantification limit was determined as 4.65µg/mL.

Reducing Power Assay:-

Reducing power assay is a convenient and rapid screening method for measuring the antioxidant potential (Koleva II 2002). The reduction ability ("Fe^{3+} to Fe^{2+} transformation" in terms of increasing absorbance) was found to increase with rising concentration in all the samples. 60 µg/mL of the sample was shown to have maximum reducing power (absorbance ~1.58), which was much greater than that of Gallic acid (considering the amount of phenolics present in the samples) which was used as positive control which gave maximum absorbance at a concentration of 100µg/mL . [Table 2]

Free radical scavenging activity:

Free radical scavenging action is considered to be one among the various mechanisms for antioxidant activity. The free radical scavenging activity of the formulation was studied by testing its ability to bleach the stable DPPH radical. This method is based on the reduction of alcoholic DPPH solution in the presence of hydrogen donating antioxidant (AH) due to the formation of non-radical form DPPH-H by the reaction DPPH + AH → DPPH-H + A. The remaining DPPH measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [(Navarro CM 1993), (Bagul MS 2003)]. This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517 nm (violet colour) and when it is quenched by the extract, there is a decrease in absorbance. [Table-3]
The percentage Antioxidant activity (% AA) was measured by the formula.

% AA= (Ac-At) ×100/Ac.
Ac is the absorbance of control
At is the absorbance of test sample.

DPPH assay confirmed that formulation is highly reactive and maximum antioxidant activity 75.50% was shown by 20ppm solution of the formulation. This is much better antioxidant than the standard Ascorbic acid. The IC_{50} value for the formulation was found to be 8.5 µg as compared to that for Ascorbic acid i.e. 22.5µg.

Statistical analysis
Three batches of the formulation (same company) were analyzed. All the analyses were performed with the aqueous and alcoholic extracts of the formulation. All estimations were repeated thrice and the results expressed as mean± standard deviation.

CONCLUSION
The formulation 'Vizhal Veradi Kashayam' was found to possess high free radical scavenging activity and reducing potential. High reduction potential indicates the redox property of the formulation, which allows it to act as antioxidant. The formulation is found to have high phenolic content too. It is reported that that plant metabolites like flavonoids, tannin, catechins and other Phenolics compounds possess antioxidant activity (Rice-Evan 1995) and played a preventive role in the development of cancer, heart and age related diseases. They have also been reported to be chemo preventive agents by lowering and repairing damaged cells. The formulation 'Vizhal Veradi Kashayam' possessing high phenol content also showed high antioxidant activity and reducing

power. Therefore isolation and quantification of these bioactive molecules could find numerous applications in the treatment of neurodegenerative diseases.

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Table 1. Calibration curve for Gallic acid

No.	Concentration Of Gallic acid (µg/mL)	Absorbance
1	20	0.071±0.002
2	40	0.137±0.002
3	60	0.199±0.010
4	80	0.273±0.009
5	100	0.336±0.013
6	120	0.402±0.017
7	140	0.473±0.020

Table 2. Reducing power assay of *the formulation*

Measured by the transformation of Fe (III) to Fe (II).

No.	Concentration (µg/mL)	Absorbance of	
		Gallic acid	Formulation
1	10	0.220±0.010	0.371±0.0104
2	20	0.330±0.008	0.753±0.0103
3	40	0.581±0.012	0.901±0.113
4	60	0.902±0.011	1.580±0.132
5	80	1.094±0.009	.---
6	100	1.151±0.0131	.---

Table 3. Antioxidant Activity (% AA) of Formulation.

No.	Concentration In ppm.	Percentage inhibition(AA%) of	
		Standard	Formulation.
1	5	12.12±0.8	32.65±1.02
2	10	23.32±1.1	56.12±2.10
3	15	31.20±1.2	66.33±1.35
4	20	44.44±1.0	75.51±1.51
5	25	55.55±1.3	75.56±1.56
6	30	67.67±2.0	---
7	35	73.73±2.1	---
8	40	82.82±1.86	----
9	45	88.88±2.32	----

Fig. 1. Callibration curve for Gallic acid.

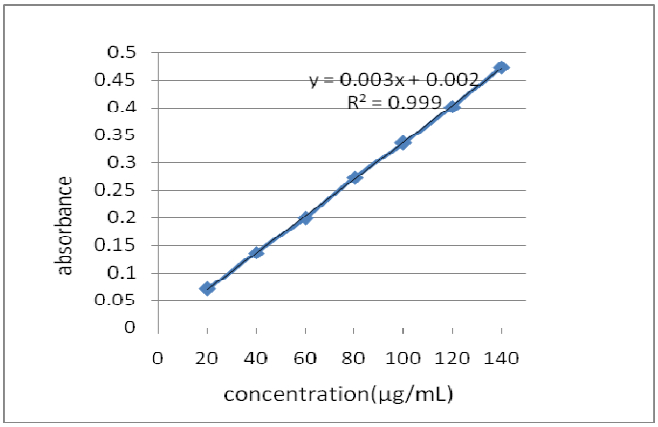

Fig. 2. A Graph of Antioxidant Activity against Concentration in ppm.