Research Paper www.ijcps.com

International Journal of
Chemical and Pharmaceutical Sciences
2012, Dec., Vol. 3 (4)

ISSN: 0976-9390
IJCPS

In vitro antioxidant potential of *Cynoglossum zeylanicum* (Vahl ex Hornem) Thunb.ex Lehm. whole plant (Boraginaceae)

[1]Anitha M, [2] Sakthidevi G, [3] Muthukumarasamy **S** and [2] Mohan VR*

[1] Department of Botany, Sri Parasakthi Collge for Women, Courtallum, Tamil Nadu. India.

[2] Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu, India.

[3] Department of Botany, Sri K.G.S.Arts College, Srivaikuntam, Tamil Nadu, India.

*Corresponding Author: E-Mail: vrmohanvoc@gmail.com

ABSTRACT

Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of the *Cynoglossum zeylanicum* whole plant have been tested using various antioxidant model systems viz., DPPH, hydroxyl, superoxide and ABTS. Ethanol extract of *Cynoglossum zeylanicum* is found to possess highest DPPH radical scavenging activity followed by methanol and ethyl acetate extracts. The ethyl acetate extract of *Cynoglossum zeylanicum* exhibits potent hydroxyl radical scavenging activity. The superoxide radical scavenging activity of *Cynoglossum zeylanicum*, benzene extract records the highest. With respect to ABTS radical cation scavenging, methanol, ethanol and ethyl acetate extracts of *Cynoglossum zeylanicum* present high activities compared with other solvent extracts. Like the antioxidant activity, reducing power of the extract increases with increase in concentration. This study indicates significant free radical scavenging potential of *Cynoglossum zeylanicum* whole plant which can be exploited for the treatment of various free radical mediated elements.

Key words: *Cynoglossum zeylanicum,* Antioxidant, DPPH, ABTS, Methanol.

1. INTRODUCTION

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neuro-degeneration, Parkinson's diseases, mongolism, ageing process and perhaps dementias[1]. Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing. However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis[2]. There is growing interest toward natural antioxidants from herbal sources[3-5]. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems [6-8].

However, the use of natural antioxidants is limited by a lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data[9]. Natural antioxidants tend to be safer and they also possess anti-viral, anti-inflammatory, anti-cancer, anti-tumor and hepatoprotective properties[10]. Therefore, the evaluation of antioxidant activity of various plant extract is considered as an important step in the identification of their ability to scavenge the free radicals.

Cynoglossum zeylanicum belongs to Boraginaceae family. It is commonly known as "Jathakkai". Decoction prepared from the whole plant is used to arrest vomiting by Badaga community in Nilgiri Biosphere Reserve, Tamil Nadu.

However, no systematic attempts have been made to establish scientific basis of beneficial effects of *Cynoglossum zeylanicum* whole plant extracts. To our knowledge, no reports on the *in vitro* antioxidant activity of *Cynoglossum zeylanicum* whole plant. This study was therefore undertaken to evaluate the effect of different extracts of whole plant of *Cynoglossum zeylanicum* on *in vitro* antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant material

Whole plant of *Cynoglossum zeylanicum* (Vahl ex Hornem) Thunb.ex Lehm was collected

from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

2.2. Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method[11]. Samples (100µL) were mixed thoroughly with 2 mL of 2% Na_2CO_3. After 2 min. 100 µL of Folin-Ciocalteu reagent was added to the mixture. The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was measured at 743 nm against a blank. Total phenolic content was expressed as gram of gallic equivalents per 100 gram of dry weight ($g100g^{-1}DW$) of the plant samples.

2.3. Estimation of Flavonoids

The flavonoids content was determined according to Eom *et al* [12]. An aliquot of 0.5mL of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

2.4. DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H[13].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [13]. Briefly, an 0.1mm solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts in methanol at different concentration (50, 100, 200, 400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

2.5. Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of[14]. Stock solutions of EDTA (1mM), FeCl3 (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$,0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50, 100, 200, 400 & 800µg/mL) dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^0c for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The hydroxyl radical scavenging activity of the extract is reported as % inhibition of deoxyribose degradation is calculated by using the following equation

Hydroxyl radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.6. Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski[15]. The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, P^H 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration(50, 100, 200, 400 & 800µg/mL) and 0.5 mL Tris – HCl buffer (16mM, P^H 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^oC for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by using the following equation

Superoxide radical scavenging activity= $\{(A_0 - A_1)/A_0)*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of

all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.7. Antioxidant Activity by Radical Cation (ABTS +)

ABTS assay was based on the slightly modified method of Re *et al* [16]. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

ABTS radical cation activity = $\{(A_0 - A_1)/A_0\}*100\}$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

2.8. Reducing Power

The reducing power of the extract was determined by the method of Singh *et al* [17] with minor modification to Oyaizu[18]. 1.0 mL of solution containing 50, 100, 200, 400 & 800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0 mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

3. RESULTS

3.1. Total phenolic content and total flavonoid conent

The total phenolic content and total flavonoid content of the methanol extract of *Cynoglossum zeylanicum* whole plant was found to be 0.48g100g^{-1} and 0.64g100g^{-1} respectively.

3.2. DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Cynoglossum zeylanicum* whole plant was shown in Fig 1. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, ethanol extract exhibited highest DPPH radical scavenging activity. At 800µg/mL concentration ethanol extract of *Cynoglossum zeylanicum* possessed 118.51% scavenging activity on DPPH.

Fig -1: DPPH radical scavenging activity of different solvent extracts of whole plant of *Cynoglossum zeylanicum*

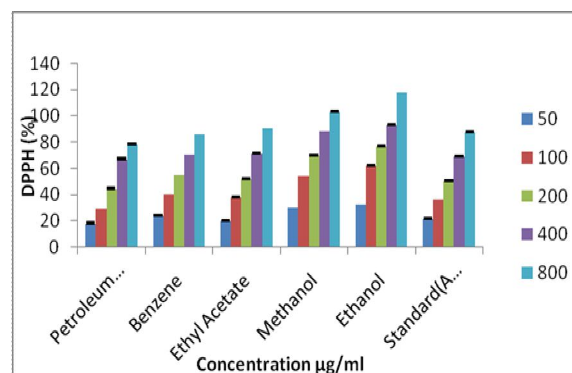


Fig -2: Hydroxyl radical scavenging activity of different solvent extracts of whole plant of *Cynoglossum zeylanicum*

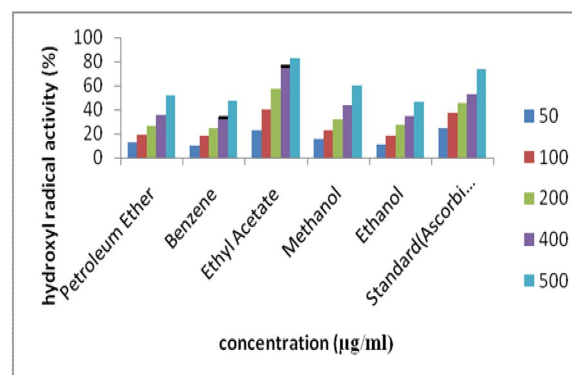


Fig -3: Superoxide radical scavenging activity of different solvent extracts of whole plant of *Cynoglossum zeylanicum*

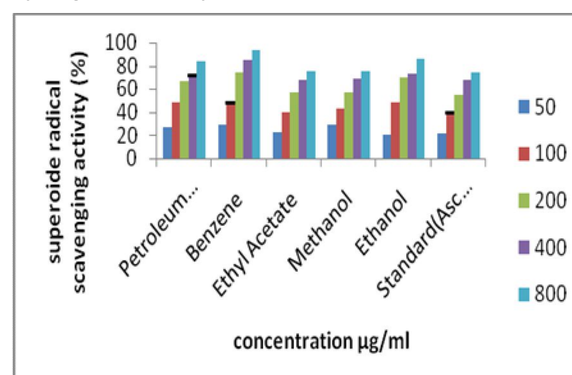


Fig -4: ABTS radical cation scavenging activity of different solvent extracts of whole plant of *Cynoglossum zeylanicum*

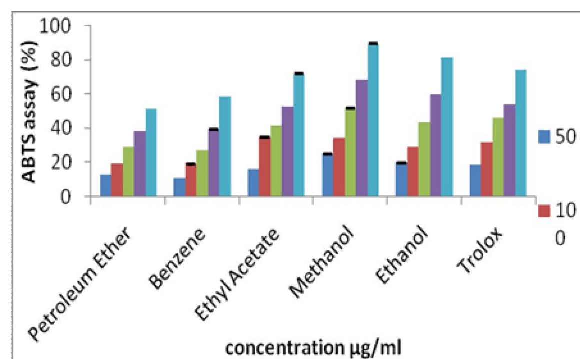
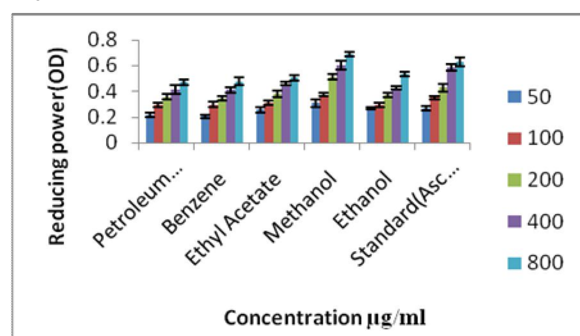


Fig -5: Reducing power ability of different solvents extract of whole plant of *Cynoglossum zeylanicum*



3.3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Cynoglossum zeylanicum* whole plant was shown in Fig 2. Ethyl acetate extract showed very potent activity. At 800µg/mL concentration, *Cynoglossum zeylanicum* possessed 83.14% scavenging activity on hydroxyl radical.

3.4. Superoxide radical scavenging activity

The *Cynoglossum zeylanicum* whole plant extracts were subjected to be superoxide scavenging assay and the results were shown in Fig 3. It indicates that benzene extract of *Cynoglossum zeylanicum* whole plant (800µg/mL) exhibited the maximum superoxide scavenging activity of 93.61% which is higher than the standard ascorbic acid whose scavenging effect is 74.91%µg/mL.

3.5. ABTS radical cation scavenging activity

The *Cynoglossum zeylanicum* whole plant extracts were subjected to be ABTS radical cation scavenging activity and the results were shown in Fig 4. The methanol extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/mL concentration, *Cynoglossum zeylanicum* whole plant possessed 84.63% scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 74.39%.

3.6. Reducing power

Figure 5 showed the reducing ability of different solvent extracts of *Cynoglossum zeylanicum* whole plant compared to ascorbic acid. Absorbance of the solution was increased when the concentration increased. A higher absorbance indicates a higher reducing power. Among the solvent tested, methanol extract exhibited higher reducing activity.

3.7. IC_{50} value

IC_{50} values of petroleum ether extract of *Cynoglossum zeylanicum* whole plant and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging activity and trolox for ABTS radical cation scavenging activity were found to be 17.93µg/mL and 20.19µg/mL; 15.63µg/mL and 20.22µg/mL; 26.11µg/mL and 23.88µg/mL and 18.28µg/mL and 21.63 µg/mL respectively. IC_{50} values of ethyl acetate extract of *Cynoglossum zeylanicum* whole plant and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 20.56µg/mL and 20.19 µg/mL; 22.53µg/mL and 20.22µg/mL; 24.89µg/mL and 23.88µg/mL and 20.22 µg/mL and 21.63µg/mL respectively. IC_{50} values of methanol extract of *Cynoglossum zeylanicum* whole plant and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 28.33µg/mL and 20.19µg/mL; 18.55µg/mL and 20.22µg/mL; 25.08µg/mL and 23.88 µg/mL and 26.89µg/mL and 21.63µg/mL respectively. IC_{50} values of ethanol extract of *Cynoglossum zeylanicum* whole plant and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 29.56µg/mL and 20.19µg/mL; 14.34µg/mL and 20.22µg/mL; 27.69µg/mL and 23.88µg/mL and 21.63µg/mL and 21.63µg/mL respectively (Table 1).

Table-1: IC_{50} values of different solvent extract of *Cynoglossum zeylanicum* whole plant

Different solvent extract	DPPH assay	Hydroxyl assay	Superoxide dismutase activity	ABTS assay
Petroleum ether	17.93	15.63	26.11	4.39
Benzene	18.32	15.09	30.64	16.28
Ethyl acetate	20.56	22.53	24.89	20.22
Methanol	28.33	18.55	25.08	26.89
Ethanol	29.56	14.34	27.69	21.63
Standard (Ascorbicacid)	20.19	20.22	23.88	-
Standard (Trolox)	-	-	-	21.63

4. DISCUSSION

The systematic literature collection, pertaining to this investigation indicates that the plant phenolics constitute one of the major groups of compounds using as primary antioxidants or free radical scavengers. Therefore, it is necessary to determine the total amount of phenols and flavonoids in the plant extract chosen for the study. flavonoids are the most diverse and widespread group of natural compounds and are likely to be the most important natural phenolics. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging activity.

Flavonoids are important secondary metabolites of plant modulating lipid peroxidation involved in atherogenesis, thrombosis and carcinogenesis. It has been confirmed that pharmacological effects of flavonoids is correlating with their antioxidant activity[19]. Phenolic compounds are considered to be the most important antioxidants of plant materials. They contribute one of the major groups and compounds acting as primary antioxidants or free radical terminators. Antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms to free radicals. In addition, they possess ideal structural properties for free radical scavenging properties[20]. The presence of these compounds such as total phenolics and flavonoids in *Cynoglossum zeylanicum* extract may give credence to its local usage for the management of oxidative stress induced ailments.

Free radicals and other reactive species are thought to play an important role in many human diseases. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. Many secondary metabolites which include flavonoids, phenolic compounds, etc. serve as sources of antioxidants and do scavenging activity[21,22]. In this study, it is evident that the extract of the study species, *Cynoglossum zeylanicum* possess effective antioxidant activity. This feature perhaps due to the presence of respective phytochemicals like flavonoids, phenolics, etc. in this species[23].

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *Cynoglossum zeylanicum* were investigated in the present study by DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities. These methods have proven the effectiveness of the extracts in comparison to that of the reference standard antioxidants, ascorbic acid and trolox.

DPPH assay is the most widely reported method for screening antioxidant activity of many plant drugs, based on the reduction of methanolic solution of colored free radical DPPH by free radical scavenger. The procedure involves measurement of decrease in absorbance of DPPH at its absorption maxima of 516nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. DPPH is a stable, nitrogen-centered free radical which produces violet color in ethanol solution. It was reduced to a yellow colored product, diphenylpicrylhydrazine, with the addition of *Cynoglossum zeylanicum* in a concentration-dependent manner[24]. Among the solvent tested, ethanol extract of *Cynoglossum zeylanicum* whole plant exhibited more DPPH radical scavenging activity.

Hydroxyl radicals are major active oxygen species causing lipid peroxidation and enormous biological damage. Hydroxyl radical scavenging capacity of *Cynoglossum zeylanicum* extract is directly related to its antioxidant activity. This method involves *in vitro* generation of hydroxyl radicals using Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid[25]. When *Cynoglossum zeylanicum* extract was added to the reaction mixture, it removed the hyxdroxyl radicals from the sugar and prevented the reaction. Among the solvent tested, ethyl acetate possessed more hydroxyl radical scavenging activity when compared with standard ascorbic acid.

Superoxide anion is also very harmful to cellular components and produced from molecular oxygen due to oxidative enzyme of body as well as via non-enzymatic reaction such as autoxidation by catecocholamines[26]. The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560nm, *Cynoglossum zeylanicum* extract indicated ability to quench superoxide radicals in the reaction mixture. The present study showed potent superoxide radical scavenging activity for *Cynoglossum zeylanicum* whole plant extract. Benzene extract showed potent superoxide radical scavenging activity with IC_{50} value 30.64µg/mL compared to ascorbic acid 23.88µg/mL.

ABTS radical scavenging activity is relatively recent one, which involves a more drastic radical, chemically produced and is often used for screening complex antioxidant mixtures such as plant extracts, beverages and biological

fluids. The ability in both the organic and aqueous media and the stability in a wide pH range raised the interest in the use of ABTS+ for the estimation of antioxidant activity[27]. The present study, ethanol extract of whole plant of *Cynoglossum zeylanicum* were fast and effective scavengers of ABTS radical and this activity was higher than that of trolox standard. Proton radical scavenging is an important attribute of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals[28].

Several reports indicated that the reducing power of bioactive compounds was associated with antioxidant activity[29]. Therefore, it is necessary to determine the reducing power of phenolic constituents contained in the plant extracts to elucidate the relationship between their antioxidant effect and their reducing power. In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of *Cynoglossum zeylanicum* extract in concentration dependent manner when compared to the standard ascorbic acid.

The present study reveals that the whole plant of *Cynoglossum zeylanicum* exhibits satisfactory scavenging effect in all the radical scavenging assays. This is the first report on the antioxidant property of this plant. It is reported that total phenolics and flavonoids are natural products which have been shown to possess various biological properties related to antioxidant mechanisms [30]. Thus in the present study, the antioxidant potential of *Cynoglossum zeylanicum* may be attributed to the presence of flavonoids, phenolics and other constituents present them.

ACKNOWLEDGEMENT

The authors are thankful to Dr.R.Sampathraj, Honorary Director, Dr.Samen Clinical Research Laboratory, Thiruppur for providing necessary facilities to carry out this work

5. REFERENCES

1. Oke JM and Hamburger MO. Screening of Some Nigerian Medicinal Plants for antioxidant activity using 2,2-Diphenyl-Picryl-Hydrazyl Radical. Afr. J. Bio. Res., 2002; 5: 77-79.
2. Atiqur Rahman M, Mizanur Rahman, Shabah Mohammad Shadli and Alam MF. Free radical scavenging activity and phenolic conent of *Cassia sophera* L. Afr. J. Biotech., 2008; 7: 1591-1593.
3. Larson RA. The antioxidants of higher plants. Phytochemistry., 1998; 27: 969-978.
4. Gazzani G, Papetti A, Mussolini G and Daglia M. Anti and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. J. Agric. Food Chem., 1988; 46: 4118-4122.
5. Velioglu YS, Mazza G, Gao L and Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. J. Agric. Food Chem., 1988; 46: 4113-4117.
6. Cao G, Sofic ER and Prior RL. Antioxidant capacity of tea and common vegetables, J. Agric. Food Chem., 1996; 94: 3426-3431.
7. Black G and Patterson B. Fruits, vegetables and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer., 1992; 18: 1-29.
8. Ness AR and Powles JW. Fruit and vegetables and cardiovascular disease. Int. J. Epidemol.,1997; 6:1-13.
9. Shahidi F and Wanasundara UN. Natural antioxidant from low pungency mustard flour. Food Res. Int., 1994; 27: 489-493.
10. Lim YY and Murtijaya J. Antioxidant properties of *Phyllanthus amarus* as affected by different drying methods. Lebensm Wiss Technol., 2007; 40: 1664-1669.
11. Yu L, Haley S, Perret J, Harris M, Wilson J and Qian M. Free radical scavenging properties of wheat extracts. J Agri Food Chem., 2002; 50: 1619-1624.
12. Eom SH, Cheng WJ, Hyoung JP, Kim EH, Chung MI, Kim MJ, Yu C and Cho DH. Far infra red ray irradiation stimulates antioxidant activity in *Vitis flexuosa* Thunb. Berries. Kor J Med Crop Sci., 2007; 15: 319-323.
13. Blois MS. Antioxidant determination by the use of a stable free radical. Nat., 1958; 181: 1199-1200.
14. Halliwell B, Gutteridge JMC and Aruoma OI. The deoxyribose method: a simple test to be assay for determination of rate constants for reaction of hydroxyl radicals. Ana Biochem., 1987; 165: 215-219.
15. Robak J and Gryglewski RJ. Flavonoids are scavenges of superoxide anions. Biochem Pharmacol., 1988; 37: 837-841
16. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M and Rice-Evans E. Antioxidant activity applying an improved ABTS radical cation decolonization assay. Free Radical Bio Med., 1999; 26: 1231-1237.

17. Singh R, Singh B, Singh S, Kumar N, Kumar S and Arora S. Investigation of ethyl acetate extract/fractions of *Acacia nilotica* wild. Ex. Del as potent antioxidant. Rec Nat Prod., 2009; 3: 131-138.
18. Oyaizu M. Studies on product of browning reaction prepared from glucose amine. Jpn J Food Chem., 1986; 44: 307-315.
19. Sri J, Yu J, Pohorly J, Young C, Bryan M and Wu J. Optimization of the extraction of polyphenolics from grapes seed and by aqueous ethanol solution. Food Agric. Erviron., 2006; 1: 42-47.
20. Sulaiman SF, Yusoff NAM, Eldeen IM, Seow EM, Sajak AAB and Supriatno OKL. Correlation between the total phenolic and mineral contents unit antioxidant activity of eight Malaysian Bananas (Musa sp). J. Food Compost Anal., 2011; 24: 1-10.
21. Ghasemi K, Ghaseni Y and Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci., 2009; 22: 277-281.
22. Doss A, Pugalenthi M, Rajendrakumar D and Vadivel V. Phenols, flavonoids and antioxidant activity of underutilized legume seeds. Asian J Exp Biol Sci., 2010; 1: 700-705.
23. Anandakumar AM, Paulsamy S, Sathishkumar P, Senthilkumar P. Preliminary phytochemical studies for the quantification of secondary metabolites of medicinal importance in the plant, *Acalypha fruticosa* Forssk. J Appl Nat Sci., 2009: 1: 41-43.
24. Jothy SL, Zuraini Z, Sasidharan S. Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitory activities of *Cassia fistula* seeds extracts. J Med Plant Res., 2011; 5: 1941-1947.
25. Battu GR, Ethadi SR, Veda PG, Swathi PK, Chandrika K and Rao VA. Evaluation of antioxidant and anti-inflammatory activity of *Euphorbia heyneana* Spreng. Asian Pac J Trop Biomed., 2011; S191-S194.
26. Naskar S, Islam A, Mazuumder UK, Saha P, Haldar PK and Gupta M. *In vitro* antioxidant potential of hydromethanolic extract of *Phoenix dactylifera* fruits. J Sci Res., 2010; 2: 144-157.
27. Huang MH, Huang SS, Wang RS, Wu CH, Sheu MJ and Hou WC. Antioxidant and anti-inflammatory properties of *Cardiospermum halicacabum* and its reference compounds *ex vivo* and *in vivo*. J Ethnopharmacol., 2011; 133: 743-750.
28. Adedapo AA, Jimoh FO, Afolayan AJ and Masika PJ. Antioxidant properties of the leaves and stems of *Celtis Africana*. Rec Nat Prod., 2009; 3: 23-31.
29. Siddhuraju P, Mohan PS and Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves. Flowers and fruit pulp. Food Chem., 2002; 79: 61-70.
30. Shirwaikan A, Rajendran K and Dinesh K. *In vitro* antioxidant studies of *Annona squamosa*. Indian J Exp Biol., 2004; 142: 803.