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A Study on the Free Radical Scavenging Activity of Desmodium Triquetrum

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Abstract: ***The human body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally produced , or externally supplied through foods or supplements.These antioxidants act as free radical scavengers by preventing and repairing damages caused by the pro-oxidants such as Reactive Oxide Species (ROS) and therefore can enhance the immune defense and lower the risk of degenerative and other stress diseases.The present study ascertained whether the leaf extract of Desmodium triquterum could have the free radical scavenging properties.Ethyl acetate extract of Desmodium triquetrum shows more percentage of free radical inhibition than methanol extract. In DPPH, hydroxyl and superoxide radical scavenging assays, the superoxide radical scavenging assay shows highest percentage of inhibition than others in the order of Superoxide> hydroxyl > DPPH respectively.Thus leaf extract of Desmodium triquetrum has significant free radical scavenging activity.***
Keywords: Antioxident, Superoxide, DPPH, Desmodium, Soxhlet,EDTA, Methanol, Ethyl acetate

I. INRODUCTION

Antioxidants act as free radical scavengers by preventing and repairing damages caused by the pro-oxidants such as Reactive Oxide Species (ROS) and therefore can enhance the immune defense and lower the risk of degenerative and other stress diseases.Many Indian medicinal plants are considered a potential source of antioxidant compounds.Desmodium is a genus in the flowering plant of family fabaceae. These are mostly inconspicuous legumes; few have bright or large flowers, herbs or small shrubs, fruit are loments. Some Desmodium species have been shown to contain high amounts of tryptamine alkaloids, though many tryptamine-containing Desmodium species have been transferred to other genera. DMT and 5 MeO DMT occur in all green parts of D.giganteum, as well as the roots. D.triflorum roots contain DMT-N oxide. Here we sought to examine the antioxidant activity of a species of Desmodium triquetrum using methanol and ethyl acetate extracts of plant for measuring superoxide,hydroxyl and DPPH scavenging activities.

II. MATERIALS AND METHODS

The objective of the present study was to evaluate the free radical scavenging activity of Desmodium triquetrum extract. This was determined by three different strategies-hydroxyl scavenging assay, superoxide scavenging assay and DPPH scavenging assay.

A. Collection Of Plant

The leaf of plant used in this study was collected from the forests of Wayanad district, Kerala India.

B. Preparation of extract

The leaves of Desmodium triquetrum were dried under shade and ground to a powder (7.0g) which was extracted by ethyl acetate (60-80°C) in a Soxhlet apparatus for 4 hours. The extract was concentrated evaporation and dried at room temperature. The brownish

extract was resinous. Methanol extract was also prepared in similar manner. various qualitative tests were performed using these extract. All chemicals used were of analytical grade.

C. Dpph Radical Scavenging Assay

The scavenging ability of the natural antioxidants of the alcohol and acetone extracts of the rhizome towards the stable free radical DPPH was measured by the method of Mensor *et al*. (2001).

To 1.5ml of methanolic solution of DPPH, 0.06 ml of the rhizome extract dissolved in ethanol was added at different concentrations.1.44 ml of methanol was also added. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the rhizome extracts, served as the control. . After 30 minutes of incubation, the discoloration of the purple colour was measured at 518 nm in a spectrophotometer.

The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = \frac{A518\,(\text{sample}) - A518\,(\text{blank})}{A518\,(\text{blank})} \times 100$$

D. Hydroxyl Radical Scavenging Activity

The extent of the hydroxyl radical scavenging effect was assayed according to the method of Elizabeth and Rao (1990

Stock solutions of EDTA (1 mM), FeCl3 (10 mM), ascorbic acid (1 mM), H2O2 (10 mM) and deoxyribose (10 mM) are prepared in distilled deionized water. The assay can be performed by adding 0.1 ml EDTA, 0.01 ml of FeCl3, 0.1 ml of H2O2, 0.36 ml of deoxyribose, 1.0 ml of extract dissolved in distilled water, 0.33 ml of phosphate buffer (50 mM, pH7.4) and 0.1 ml of ascorbic acid in sequence. The mixture is incubated at 37°C for 1 hour. A 1.0 ml portion of the incubated mixture was mixed with 1.0 ml of 10 g/100 g TCA and 1.0 ml of 0.5 g/100 g TBA (in 0.025 M NaOH containing 0.025 g/100 g TBA) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as percentage inhibition of deoxyribose degradation (Halliwell et .al).}

The reaction mixture contains 0.1ml of deoxyribose, 0.1ml of FeCl3, 0.1ml of EDTA, 0.1ml of ascorbate, 0.1ml of KH2PO4 – KOH buffer and 0.02ml of various concentrations of extract in final volume of 1ml. The mixture was incubated at 37°C for 1 hour. At the end of the incubation period, 1.0 ml of TBA was added and heated at 95°C for 20 minutes to develop the colour. After cooling, the TBARS formation was measured spectrophotometrically at 532 nm against the blank.

E. Superoxide Scavenging Activity

The superoxide scavenging ability of the extracts was assessed by the method of winterbourne et.al. (1975).

This assay is based on the inhibition of production of nitro blue tetrazolium formazon of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560 nm. Phosphate buffer (0.067M, Ph 7.6).

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the leaf extracts (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer. The tubes were illuminated using a lamp for 30 minutes. The absorbance was measured again at 560 nm.The differences in absorbance before and after illumination was indicative of superoxide anion scavenging activity.

III. RESULTS AND OBSERVATIONS

Dpph Radical Scavenging Activity Of Ethyl Acetate Extract *Of*
Desmodium Triquetrum.

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.55	0.53	0.54	0.54	0.01	-	-
0.003	0.53	0.52	0.53	0.53	0.01	0.01	2
0.033	0.38	0.37	0.39	0.38	0.01	0.16	30
0.333	0.22	0.24	0.20	0.22	0.02	0.32	59

Desmodium Triquetrum

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.55	0.53	0.54	0.54	0.01	-	-
0.003	0.53	0.52	0.53	0.53	0.01	0.01	2
0.033	0.38	0.37	0.39	0.38	0.01	0.16	30
0.333	0.22	0.24	0.20	0.22	0.02	0.32	59

Superoxide Scavenging Activity Of Methanol Extract *Of*
Desmodium Triquetrum

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.25	0.22	0.23	0.25	0.01	-	-
0.003	0.16	0.15	0.16	0.14	0.01	0.11	44
0.033	0.12	0.11	0.10	0.12	0.01	0.13	52
0.333	0.10	0.11	0.10	0.10	0.01	0.15	59

Superoxide Scavenging Activity Of Ethyl Acetate Extract *Of*
Desmodium triquetrum

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.25	0.24	0.23	0.25	0.02	-	-
0.003	0.14	0.13	0.14	0.16	0.03	0.09	37
0.033	0.11	0.10	0.10	0.13	0.03	0.12	49
0.333	0.10	0.11	0.11	0.10	0.02	0.15	59

Hydroxyl Scavenging Activity Of Ethyl Acetate Extract *Of*

Desmodium Triquetrum

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.22	0.25	0.26	0.24	0.02	-	-
0.003	0.2	0.22	0.19	0.20	0.02	0.04	16
0.033	0.18	0.5	0.13	0.15	0.03	0.09	37
0.333	0.14	0.12	0.15	0.14	0.02	0.11	44

Hydroxyl Scavenging Activity Of Methanol Extract *Of*
Desmodium Triquetrum

EXTRACT CONCENTRATIONS (µg/ml)	ABSORBANCE READINGS AT 518nm			AVERAGE READING	SD	FINAL ABSORBANCE (DMSO-TEST)	%INHIBITION
	1	2	3				
DMSO	0.22	0.22	0.25	0.23	0.02	-	-
0.003	0.18	0.19	0.16	0.18	0.02	0.05	23
0.033	0.16	0.17	0.16	0.16	0.01	0.07	29
0.333	0.14	0.12	0.13	0.14	0.01	0.10	43

IV. DISCUSSION

In the DPPH assay, the antioxidants were able to reduce the stable radical DPPH to the yellow-colored diphenyl-picryl hydrazine. The method is based on the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. There was a significant decrease in the concentration of DPPH radical due to the scavenging ability of Desmodium and the reference compounds compared to the DMSO control (Table1). Ethyl acetate extract of Desmodium shows a gradual increase in the percentage of inhibition 2%, 30% and 59% in different concentrations such as 0.003 µg/ml, 0.033 µg/ml and 0.333 µg/ml respectively(Table 1.1). Methanol extract of Desmodium also shows a gradual increase in the percentage of inhibition (6%, 28 % and 35 % respectively) at similar concentrations (Table 1.2). DPPH• radical scavenging methods is common spectrophotometric procedures for determining antioxidant capacities of components. When an antioxidant is added to the radicals, there is a degree of decolorization owing to the presence of the antioxidants, which reverses the formation of the DPPH• radical cation: DPPH• + AH → DPPH 2 +A•

Superoxide scavenging assay is based on the inhibition of the production of nitro blue tetrazolium formazon of the superoxide ion by the plant extracts and is measured spectrophotometrically at 560 nm. There is a significant decrease in the concentration of superoxide radical due to the scavenging ability of Desmodium and the reference compounds (Table 2). DMSO was used as control for radical scavenger activity.

Ethyl acetate extract of Desmodium shows a gradual increase in the percentage of inhibition in different concentrations such as 0.003 µg/ml, 0.033 µg/ml and 0.333 µg/ml and the percentage of inhibition were 37%, 49% and 59 % respectively(Table 2.1). Methanol extract of Desmodium also shows a gradual increase in the percentage of inhibition in different concentrations and the percentage of inhibition were 44%, 52% and 59 % respectively(Table 2.2).

The hydroxyl radical scavenging activity can be measured by studying the competition between deoxyribose and the plant extracts for hydroxyl radicals generated with Fe3+/ ascorbate / EDTA / H2O2 system. The hydroxyl radicals attack deoxyribose, which eventually result in TBARS formation, which can be quantified spectrophotometrically at 540 nm. In this assay there is a significant decrease in the concentration of hydroxyl radical due to the scavenging ability of Desmodium and the reference compounds (Table 3).

Ethyl acetate extract of desmodium shows a gradual increase in the percentage of inhibition in different concentrations such as 0.003

µg/ml, 0.033 µg/ml and 0.333 µg/ml and the percentage of inhibition were 16%,37 % and 44 % respectively(3.1). Methanol extract of Desmodium also shows a gradual increase in the percentage of inhibition in different concentrations such as 0.003 µg/ml, 0.033 µg/ml and 0.33 µg/ml and the percentage of inhibition were 23%, 29% and 43% respectively(3.2).

The putative hydroxyl radical is an extremely reactive and short-lived species that can hydroxylate DNA, proteins, and lipids. Therefore, the direct scavenging of the hydroxyl radical by dietary antioxidants in a biological system is unrealistic as the cellular concentration of dietary antioxidants is negligible compared with other biological molecules. The rate constants for OH reactions have been determined by pulse radiolysis through the deoxyribose method. Therefore, the ability of antioxidants to scavenge the HO radical is not unlikely to provide any protection to biological molecules as the opportunity for HO and antioxidants to react is extremely small. On the other hand, it is possible to prevent the formation of hydroxyl radicals by either deactivating free metal ions [e.g., Fe(II)] through chelation or converting H2O2 to other harmless compounds (such as water and oxygen). Catalase converts H2O2 to O2, and H2O and metal chelators bind metal ions so that they become inert toward H2O2. Thus, dietary nutrients containing metal chelators may act as preventive antioxidants. Quantifying the capacity of the photochemical in preventing hydroxyl radical formation in vitro. (Cao.G. H et .al).

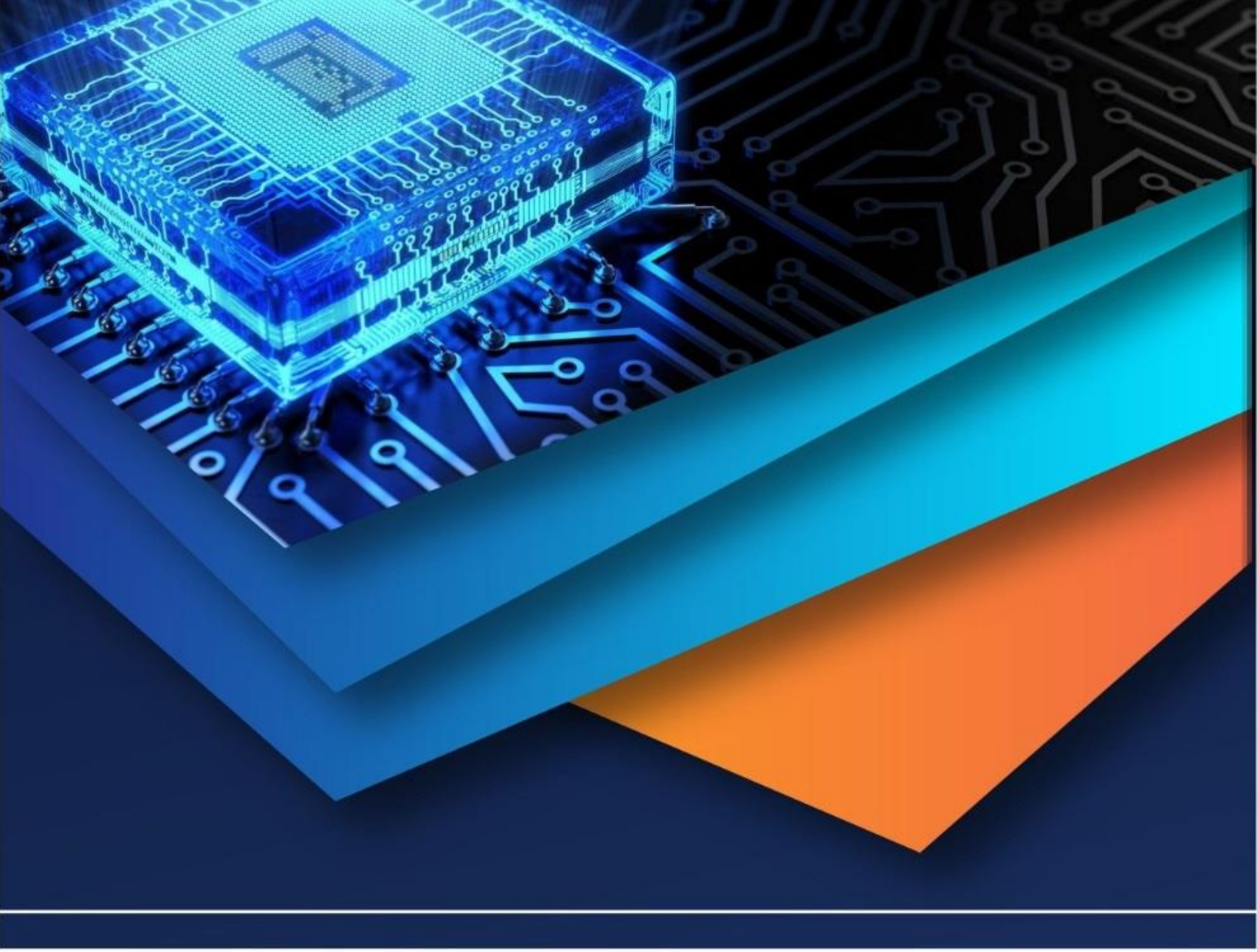
V. CONCLUSION

Desmodium species have long been used in tribal medicine to treat various ailments. Available scientific references revealed that the traditional medical uses of Desmodium species have been evaluated by modern pharmacological studies. Ethyl acetate extract of desmodium triquetrum shows more percentage of free radical inhibition than methanol extract. In DPPH, hydroxyl and superoxide radical scavenging assays, the superoxide radical scavenging assay shows highest percentage of inhibition. The different solvent plant extracts; Ethyl acetate and Methanol, tested in this study had potential Free radical scavenging activities against with the reference compounds. Our results support the use of these plants as traditional medicine and suggest that can be used as antioxidant agents in the Search of new drugs.

REFERENCES

[1] A.K.Meena et.al. (2010), Anti-inflammatory, anti-oxidant, immunomodulatory effects of desmodium ganeticum extract, Tropicaljournal of pharmaceutical Research September 2008; 7(3); (1019-1024).
[2] Acuna U.M, Atha D.E, Maj, Nee M.H & Kennelly E.J (2002), Antioxidant capacities of ten edible north American plants, Phytothen Res; 16 PP 63-65.
[3] Adams A.K and western E.O. (1999), Antioxidant vitamins and prevention of coronary heart.Biol Med -14(3), PP
[4] properties in the Ethyl acetate extract of leaves, flowers and bark by “pterospormum acerifolium.
[5] Brace, Papiya mitra, Mazumber, Dinker, Sasmal, Dhanajay Kumar, Kuhul mukhopadhyarm (2013), antioxidant properties in the Ethyl acetate extract of leaves, flowers and bark by “pterospormum acerifolium.acetate extract of leaves, flowers and bark by “pterospormum acerifolium.
[6] Dejian huang,Boxin and Ronald L(2005), The chemistry behind Antioxidant capacity assays, Agric food chem. 53, PP 1841-185
[7] F.pourmorad, S.J.Hosseini meter, N.Shahabimajd(2006),antioxidant activity, phenol and flavonoid contents of some selected Iranian and Medicinal plants African journal of Biotechnology Vol.5(1) PP-1142-1145ISSN1684-5315
[8] Halli well (2007), Dietary polyphenols; Good, bad or indifferent for our health, cardiovascular Res 73; PP 341- 347.
[9] Halli well B, Gutterridge JM, Aruoma (1987), The deoxyrobose methed : asimble “test-tube”assay for determination of rate nstants for reactions of hydroxyl radicals, Anal biochem, 165(1) : 215-9 , PMID: 312062
[10] Halvorsen BL, Rasleensudhan, Mudhulika, Bhagat, Sabil Couta, Chitrarakha, Tshu Devi, Antioxidant activity in Cinnamomum and Cinnamamum tumala, Biological Journal of Medicine, 14Noven20B
[11] Helfand .S.L.et.al (1982), chemilumine science of human natural killer celle and the relationship between target cell binding , chemilumine science and cytolysis, J.Exp.Med, 156,PP 492-50
[12] Kaur.C and Kapoor. H.C (2002), Antioxidant activity and total phenilic content by some Asian vegetables, in J food Sci technol 37: PP 153-161
[13] Kaur.C and Kapoor. H.C (2002), Antioxidant activity and total phenilic content by some Asian vegetables, in J food Sci technol 37: PP 153-16
[14] Abulesu M.and D.Muralidhara Rao (2011), the extract of Dosmodium effects on the amelioration of AD symptoms, pharmaceutical Research Journal vol 3, PP-342-356
[15] Max Well. S.R (1995), Prospectus for the use of antioxidant the rapies, Drugs 49: PP 345- 361.
[16] McCord JM, Fridovich (1969), superoxide dismutase, An enzymatic function of erythrocuprein (hemocuprein), J Biol chem., 244(22) : 6049-5
[17] Mensor, Rajendra kshirsagar, Shakti Upadhyay(2006),Free radical scavenging of medicinal plants fromTripura,North east India (Natural Product Radiance, Vol PP 117-112).
[18] Miller and Rice – Evans (2006), comparison of ABTs, DPPH, FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts, journal of food composition and analysis 19, PP 669 – 675
[19] Prior. R.L (2003), fruits and vegetables in the prevention by cellular oxidative damage Am J clin Natr 78, PP 5705- 5785.
[20] Renaud et.al (2008), oxidative stress play an important role in development of anaemia in malaria, pharmaceutical Research; 7(3), PP 1019- 1024
[21] Rukmini M.S,Bene dicta D’souza and vivan D’souza(2004), super oxide dismutase and catalase activities their correlation with malondialdehyde in schizophrenic patients, indian journal of clinical Biochemistry.19(2)PP 114-1856

[22] Saleem A, Ahotupa M, pihlaja K (2001), total phenolic concentration & in antioxidant potential extract by medicinal plants by Pakistan, Nature foirsch 56: PP 973-978
[23] Saleem A, Ahotupa M, pihlaja K (2001), total phenolic concentration & in antioxidant potential extract by medicinal plants by Pakistan, Nature foirsch 56: PP 973-978
[24] Terao J and pistula m.K (1997), Flavonoids as inhibitators by Lipid peroxidation in membranes; flavonoids in health and disease, Marcel Decker Newyark, PP 277-295
[25] Virgili.F, Marnio.M (2008), regulation of cellular signals from nutritional Molecules; a specific Role for phytochemicals beyond antioxidant activity, (2008), free radic Biol Med 45: PP 1205-1216.



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