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IN-VITRO FREE RADICAL SCAVENGING POTENTIAL OF TRADITIONAL SIDDHA FORMULATION SERAANKOTTAI NEI BY DPPH, NO, ABTS AND HYDROGEN PEROXIDE RADICAL SCAVENGING ASSAY

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ABSTRACT

Free radical and reactive oxygen species (ROS) becomes the main cause of several diseases in humans that are generated due to imbalance between formation and neutralization of prooxidants resulting in oxidative stress. They cause oxidative damage to lipids, proteins, and DNA, eventually leading to many chronic diseases, such as cancer, diabetes, aging, and other degenerative diseases in humans along with lipid peroxidation. Oxidative stress due to the production of free radicals such as superoxide radical (O2•−), hydroxyl radical (•OH), peroxide radical (ROO•), and nitric oxide radical, is the major cause of a variety of pathological conditions including coronary heart diseases, reperfusion injury, inflammation, drug toxicity and neurodegenerative diseases. There is a profound risk of using synthetic

antioxidants which are known to have side effects and have been proved carcinogenic. Thus there has been increased interest in natural antioxidants, especially those of herbal and traditional origin. It has been reported that herbal formulations, their derivatives and analogs, represent over 50% of all drugs in clinical use, in which natural products derived from higher plants represent about 25% of the total. Antioxidant substances can scavenge the harmful action of the free radicals. Several siddha formulations which are reported to possess extensive antioxidant property one among such formulation is Seraankottai Nei (SKN). The results of DPPH radical scavenging assay has shown that the percentage inhibition of DPPH radical by test drug SKN ranges from 9.08 to 52.87%. Similarly the NO radical scavenging activity of the SKN ranges from 44.74 to 90.64%. ABTS radical scavenging activity of SKN was found to be 10.5 to 68.9% and with hydrogen peroxide radical scavenging activity it was from 5.85 to 54.96%. Based on the results obtained from the In-vitro anti-oxidant assay of the drug SKN it was concluded that the siddha formulation SKN has promising anti-oxidant activity and may be used for management of stress related disorders further detailed preclinical investigation has to be carried out to ensure the in-vitro data's before prescribing the same for clinical application.

KEYWORDS: Free radical, ROS, DPPH, ABTS, NO, Hydrogen peroxide, Siddha, Seraankottai Nei.

1. INTRODUCTION

A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants.[1] The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids.[2]

Oxidative stress (OS) occurs when the production of damaging free radicals exceeds the capacity of the body's antioxidant defenses to detoxify them. Thus, oxidative/nitrosative stress is a disturbance in the balance between the production of reactive oxygen

species/reactive nitrogen species (ROS/RNS) and antioxidant defenses, which may lead to tissue injury.[3,4] Overproduction of free radicals can cause oxidative damage to biomolecules, (lipids, proteins, DNA), eventually leading to many chronic diseases such as atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, cardiovascular diseases, chronic inflammation, stroke and septic shock, aging and other degenerative diseases in humans.[5-6]

Antioxidants are compounds that protect cells against the damaging effects of ROS, such as superoxide, hydrogen peroxide, singlet oxygen, peroxyl radicals, hydroxyl radicals and peroxynitrite. Some ROS, such as superoxide and hydrogen peroxide, are normally produced in cells as by-products of biochemical reactions or as signaling molecules. When ROS-generating reactions are activated excessively, pathological quantities of ROS are released to create an imbalance between antioxidants and ROS. Oxidative stress has been linked to cardiovascular disease, diabetes, pulmonary disease, cancer, and other degenerative diseases. Herbal formulations may protect against these diseases by contributing to the total antioxidant defense system of the human body. The efficacy of herbal antioxidants has been suggested in several studies.

Most of preparations belongs to Indian system of traditional siddha medicines possess majority of herbal ingredients. Herbs have indigenous potential of producing secondary metabolites. Phytocomponents derived from herbal origin are considerably act as an natural antioxidants. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Traditional medicine containing herbal components considered as good antioxidant since ancient times. The main aim of the present investigation is evaluate the Invitro antioxidant potential of the siddha formulation Seraankottai Nei and to document the research findings to as monograph for assisting the researcher who works on similar formulation in near future.

2. MATERIALS AND METHODS

2.1. Source of raw drugs

The Required raw materials were procured from a well reputed indigenous drug shop from Chennai, Tamil Nadu, India. Herbal drugs were authenticated by the Botanist, Government

Siddha Medical College, Arumbakkam, Chennai, Tamil Nadu, India. The test drug Seraankottai Nei was prepared as per siddha vaidhya thirattu.

2.2. Ingredients

1. Marking nuts purified (Suththi seitha seran kottai)– 200gms
2. Cows milk (pasum pal)- 1500 gms
3. Ghee (Nei) – 1500 gms

2.3. Formulation of Seraankottai Nei[7]

Kernels from marking nuts were removed and were boiled with 500 gms of milk for brief period of time. Decant the decoction then add 500 gms of milk to the boiled and again boil the same. Recover the decoction and repeat the process again with the remaining milk. Mix all the three portions of milk nut decoction (discarding the nuts) with the ghee and boil till dehydrated followed by filtration and storage.

2.4. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay[8]

The antioxidant activity of test drug sample SKN was determined using the 2,2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay. Sample SKN was mixed with 95% methanol to prepare the stock solution in required concentration. From the stock solution 1ml, 2ml, 4ml, 6ml 8ml and 10ml of this solution were taken in five test tubes and by serial dilution with same solvent were made the final volume of each test tube up to 10 ml whose concentration was then 10 µg/ml, 20 µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. Ascorbic acid were used as standard was prepared in same concentration as that of the test drug by using methanol as solvent. Final reaction mixture containing 1 ml of 0.3 mM DPPH methanol solution was added to 2.5 ml of sample solution of different concentrations and allowed to react at room temperature. Absorbance in the presence of test sample SKN at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100µg/ml) was noted after 15 min incubation period at 37^0C. Absorbance was read out at 517 nm using double-beam U.V Spectrophotometer by using methanol as blank.

% scavenging = [Absorbance of control - Absorbance of test sample/Absorbance of control] X 100

The effective concentration of test sample SKN required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations.

2.5. Nitric Oxide Radical Scavenging Assay[9]

The concentrations of test sample SKN are made into serial dilution from 10−100 µg/mL and the standard gallic acid. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5 mL of 10 mM sodium nitroprusside in phosphate buffered saline was mixed with 1 mL of the different concentrations of the test drug (10−100 µg/mL) and incubated at 25°C for 180 mins. The test drug SKN was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the test drug but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. The absorbance was measured at 546 nm using a Spectra Max Plus UV-Vis microplate reader (Molecular Devices, GA, USA). Gallic acid was used as the positive control. The percentage inhibition of the test drug SC and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug SC and gallic acid were calculated using the following formula:

percentage nitrite radical scavenging activity:

$$\text{nitric oxide scavenged } (\%) = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100,$$

where A_{control} = absorbance of control sample and A_{test} = absorbance in the presence of the samples extracts or standards.

2.6. ABTS Assay[10]

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug SKN against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS radicals. The ABTS radical cation method was modified to evaluate the free radical-scavenging effect of one hundred pure chemical compounds. The ABTS reagent was prepared by mixing 5 mL of 7 mM ABTS with 88 µL of 140 mM potassium persulfate. The mixture was then kept in the dark at room temperature for 16 h to allow free radical generation and was then diluted with water (1 : 44, v/v). To determine the scavenging activity, 100 µL ABTS reagent was mixed with 100 µL of test sample (10-100µg/ml) and was incubated at room temperature for 6 min. After incubation, the absorbance was measured 734 nm. 100% methanol was used as a control. Gallic acid with same concentrations of test drug SKN was measured following the same procedures described above and was used as positive controls. The antioxidant activity of the test sample SC was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

$$\text{Radical scavenging } (\%) = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

2.7. Hydrogen Peroxide Radical Scavenging Assay[11]

A hydrogen peroxide solution (2 mM) was prepared in 50 mM phosphate buffer (pH 7.4). Aliquots (0.1 mL) of the test sample SKN (different concentration ranging from 10-100µg/ml) were transferred into the test tubes and their volumes were made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4). After adding 0.6 mL hydrogen peroxide solution, tubes were vortexed and the absorbance of the hydrogen peroxide at 230 nm was determined after 10 min, against a blank. BHA was used as the positive control. The percentage inhibition of the test drug SKN and standard was calculated and recorded. The percentage radical scavenging activity of the test drug SKN and BHA were calculated using the following formula:

$$\text{Radical scavenging (\%)} = \left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100$$

3. RESULTS

3.1. Result Analysis of DPPH radical scavenging activity of SKN

The results of DPPH radical scavenging activity of the sample SKN shown that the test drug has significant radical scavenging activity dose dependently on DPPH radicals. Sample SKN exerted percentage inhibition of DPPH radical ranges from 9.08 to 52.87% when compared with standard ascorbic acid with percentage inhibition ranges from 42.54 to 97.09%. The IC50 value of the trial drug was found to be 96.23 µg /ml when compared with standard ascorbic acid with (IC_{50} value 22.04 µg/ml). The values of DPPH free radical scavenging activity of the SKN was listed and illustrated in (Table 1 and Figure 1).

Table No 1: Percentage inhibition of test drug SKN on DPPH radical scavenging assay.

Concentration (µg/ml)	% Inhibition of SKN	% Inhibition of Ascorbic Acid
10 µg/ml	9.083 ± 3.655	42.54 ± 2.068
20 µg/ml	15.72 ± 3.086	51.47 ± 4.797
40 µg/ml	21.26 ± 6.07	61.4 ± 6.106
60 µg/ml	30.72 ± 3.519	66.87 ± 6.779
80 µg/ml	46.01 ± 11.18	74.93 ± 6.144
100 µg/ml	52.87 ± 9.522	97.09 ± 2.063

Data are given as Mean ± SD (n=3).

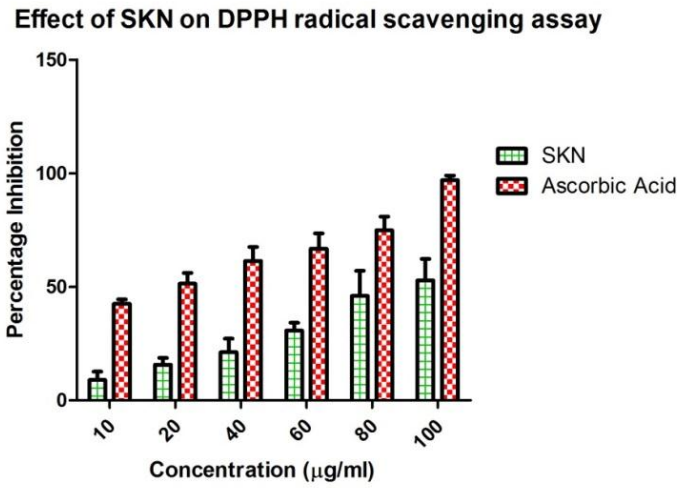

Figure 1: DPPH radical Inhibition potential of SKN and Ascorbic Acid.

3.2. Result Analysis of NO radical scavenging activity of SKN

NO radical scavenging property seems to be one of the essential qualities of the drug to be act as an effective antioxidants. Sample SKN exerted percentage inhibition of NO ranges from 8.16 to 47.56% when compared with standard gallic acid with percentage inhibition ranges from 44.74 to 90.64%. The corresponding IC50 value of the trial drug was found to be 110.34 (µg /ml) when compared with standard gallic acid with (IC_{50} value 12.29 µg/ml). The data's of NO radical scavenging activity of the SKN was listed and illustrated in (Table 2 and Figure 2).

Table No 2: Percentage inhibition of test drug SKN on Nitric Oxide radical scavenging assay.

Concentration (µg/ml)	% Inhibition of SKN	% Inhibition of Gallic Acid
10 µg/ml	8.168 ± 2.207	44.74 ± 3.775
20 µg/ml	13.5 ± 3.545	56.83 ± 2.643
40 µg/ml	19.97 ± 3.729	63.04 ± 5.669
60 µg/ml	30.27 ± 5.897	76.63 ± 3.469
80 µg/ml	39.81 ± 9.49	81.74 ± 5.411
100 µg/ml	47.56 ± 13.64	90.64 ± 1.221

Data are given as Mean ± SD (n=3).

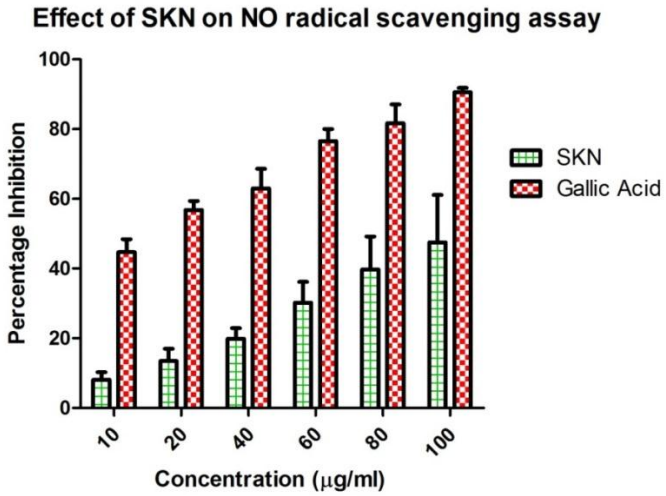

Figure 2: NO radical Inhibition potential of SKN and Gallic acid.

3.3. Result Analysis of ABTS radical scavenging activity of SKN

The results of ABTS radical scavenging activity of the sample SKN shown that the test drug has significant ABTS radical scavenging activity dose dependently. Sample SKN exerted potential ABTS scavenging property whose values radical ranges from 10.5 to 68.9% when compared with standard gallic acid with percentage inhibition ranges from 41.04 to 92.5%. The corresponding IC50 value of the trial drug was found to be 63.74 (µg /ml) when compared with standard gallic acid with (IC_{50} value 11.23 µg/ml). The values of ABTS free radical scavenging activity of the SKN was listed and illustrated in (Table 3 and Figure 3).

Table No 3: Percentage inhibition of test drug SKN on ABTS radical scavenging assay.

Concentration (µg/ml)	% Inhibition of SKN	% Inhibition of Gallic Acid
10 µg/ml	10.5 ± 1.533	41.04 ± 7.77
20 µg/ml	22.8 ± 3.431	58.62 ± 4.635
40 µg/ml	31.21 ± 2.721	69.55 ± 4.208
60 µg/ml	45.29 ± 2.366	77.58 ± 2.606
80 µg/ml	52.15 ± 2.026	84.61 ± 0.9449
100 µg/ml	68.91 ± 12.2	92.56 ± 3.268

Data are given as Mean ± SD (n=3).

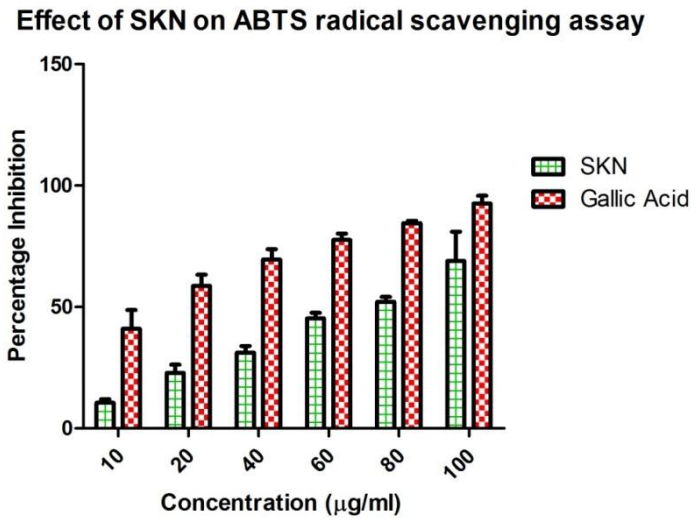

Figure 3: ABTS radical Inhibition potential of SKN and Gallic acid.

3.4. Result Analysis of Hydrogen peroxide radical scavenging activity of SKN

Siddha drug SKN were screened for hydrogen peroxide radical scavenging activity and the percentage inhibition ranges from 5.85 to 54.96% when compared with standard BHA with percentage inhibition ranges from 47.28 to 93.22%. The corresponding IC50 value of the trial drug was found to be 91.45 (µg /ml) when compared with standard BHA with (IC_{50} value 4.38 µg/ml). The values of hydrogenperoxide free radical scavenging activity of the SKN was listed and illustrated in (Table 4 and Figure 4).

Table No 4: Percentage inhibition of test drug SKN on hydrogen peroxide radical scavenging assay.

Concentration (µg/ml)	% Inhibition of SKN	% Inhibition of BHA
10 µg/ml	5.855 ± 3.767	47.28 ± 3.378
20 µg/ml	14.57 ± 4.247	59.82 ± 5.707
40 µg/ml	25.77 ± 7.065	70.09 ± 2.282
60 µg/ml	34.03 ± 5.542	77.15 ± 7.059
80 µg/ml	46.33 ± 11.6	85.2 ± 6.922
100 µg/ml	54.96 ± 10.68	93.22 ± 1.933

Data are given as Mean ± SD (n=3).

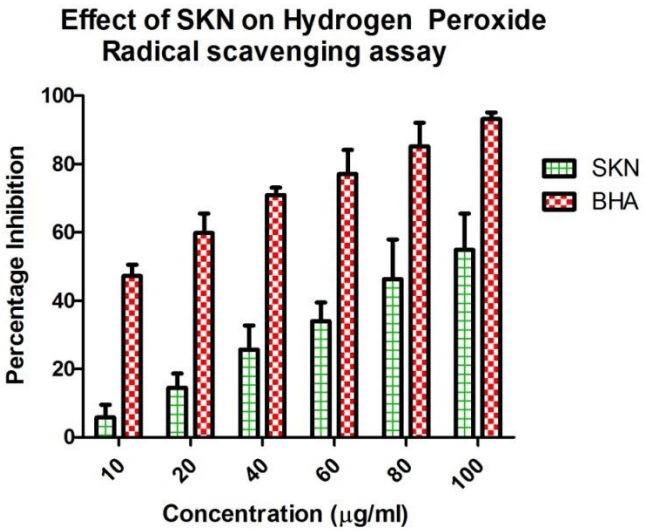

Figure 4: Hydrogen Peroxide radical Inhibition potential of SKN and BHA.

4. DISCUSSION

Oxidative stress is an imbalance between the production of ROS and the defense systems to readily detoxify them.[11] In human, oxidative damage and free radicals are associated with a number of diseases including atherosclerosis[12], Alzheimer's disease[13], cancer[14], ocular disease[15], diabetes[16], rheumatoid arthritis[17] and motor neuron disease.[18] Many studies have shown the benefit of antioxidants in the prevention or delaying the course of these diseases Natural antioxidant system is sorted in two major groups, enzymatic and non-enzymatic. Enzymatic antioxidants are comprised of limited number of proteins such as catalase, glutathione peroxidase as well as superoxide dismutase (SOD) along with some supporting enzymes. Non-enzymatic antioxidants include direct acting antioxidants, which are extremely important in defense against OS. Most of them include ascorbic and lipoic acid, polyphenols and carotenoids, derived from dietary sources. The cell itself synthesizes a minority of these molecules. Indirectly acting antioxidants mostly include chelating agents and bind to redox metals to prevent free radical generation.[19-21]

The DPPH is a stable radical with a maximum absorption at 517 nm that can readily undergo scavenging by antioxidant[22] have been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the anti-oxidative activity of herbal supplements.[23] In the present study siddha drug SKN exerted percentage inhibition of DPPH radical ranges from 9.08 to 52.87% when compared with standard ascorbic acid with percentage inhibition ranges from 42.54 to 97.09%. The IC50 value of the trial drug was

found to be 96.23 µg /ml when compared with standard ascorbic acid with (IC_{50} value 22.04 µg/ml).

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage *in vivo*.[24] Results of hydrogen peroxide radical scavenging assay of the siddha drug SKN revealed that siddha drug SKN possess the percentage inhibition ranges from 5.85 to 54.96% when compared with standard BHA with percentage inhibition ranges from 47.28 to 93.22%.

Excess NO is cytotoxic either by combining with tyrosine that is essential for catalytic function of enzyme ribonucleoside diphosphate reductase or by forming ONOO°. Excess vascular O_2° production could contribute to hypertension and vasospasm. In the present investigation it was observed that the test drug SKN exerted percentage inhibition of NO ranges from 8.16 to 47.56% when compared with standard gallic acid with percentage inhibition ranges from 44.74 to 90.64%. The corresponding IC50 value of the trial drug was found to be 110.34 (µg /ml) when compared with standard gallic acid with (IC_{50} value 12.29 µg/ml). Similarly the results of ABTS radical scavenging activity of the sample SKN shown that the test drug has significant ABTS radical scavenging activity dose dependently. Sample SKN exerted potential ABTS scavenging property whose values radical ranges from 10.5 to 68.9% when compared with standard gallic acid with percentage inhibition ranges from 41.04 to 92.5%. The corresponding IC50 value of the trial drug was found to be 63.74 (µg /ml) when compared with standard gallic acid with (IC_{50} value 11.23 µg/ml).

5. CONCLUSION

Currently, a considerable amount of research focuses on the ROS-mediated pathophysiology of different diseases. These illness cause significant patient morbidity and escalate healthcare costs. Several medications for the treatment of these diseases are believed to act through an antioxidant mechanism. It was evident from the results obtained from the present study that the siddha medicine seraankottai Nei possess significant radical scavenging activity in all the DPPH, ABTS, NO and hydrogen peroxide radical assays. Based on the results of the current investigation it was concluded the siddha drug SKN may acts as better remedy for management of stress oriented disease with necessary preclinical investigation to further strengthen the Invitro data's.

6. REFERENCES

1. Cheeseman KH, Slater TF. An introduction to free radicals chemistry. Br Med Bull, 1993; 49: 481–93.
2. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol, 2001; 54: 176–86.
3. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol, 2002; 30(6): 620–50.
4. Slater TF. Free-radical mechanisms in tissue injury. Biochem J., 1984; 222(1): 1–15.
5. Freidovich I. Fundamental aspects of reactive oxygen species, or what's the matter with oxygen? N.Y. Acad. Sci., 1999; 893: 13.
6. Yun-Zhong F, Sheng Y, Guoyao Wu. Free radicals, antioxidants, and nutrition. Nutrition, 2002; 18: 872–879.
7. K.N. Kuppusamy Mudhaliyar. Siddha Vaidya Thirattu. Siddha Medical Literature Research Centre. 2nd edition, 1998.
8. Badami, Omprakash, Dongr SH, Suresh B. *In-vitro* Antioxidant property of *Solanum Pseudocapsicum* leaf extract. *Indian J Pharmacol*, 2005; 37: 251-252.
9. B. N. Panda, A. B. Raj, N. R. Shrivastava, and A. R. Prathani, "The evaluation of nitric oxide scavenging activity of Acalypha indica Linn Root," Asian Journal Research Chemistry, 2009; 2(2): 148–150.
10. N. Pellegrini, M. Ying, and C. Rice-Evans, "Screening of dietary carotenoids and carotenoid-rich fruits extract for antioxidant activities applying 2,2′-azobis (3-ethylbenzothine-6-surfonic acid) radical cation decolorization assay," Methods in Enzymology, 1999; 299: 384–389.
11. Ruch R. J., Cheng S.-J., Klaunig J. E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, 1989; 10(6): 1003–1008.
12. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol., 2007; 39: 44–84.
13. Bagchi K, Puri S. Free radicals and antioxidants in health and disease. EMHJ., 1998; 4: 350–360.
14. Vertuani S, Angusti A, Manfredini S. The antioxidants and pro-antioxidants network: an overview. Curr Pharm., 2004; 14: 1677–1694.

15. Nunomura A, Castellani R, Zhu X, Moreira P, Perry G, Smith M. Involvement of oxidative stress in Alzheimer disease. J Neuropathol Exp Neurol., 2006; 65: 631–641.
16. Tamimi RM, Hankinson SE, Campos H, Spiegelman D, Zhang S, Colditz GA, et al. Plasma carotenoids, retinol, and tocopherols and risk of breast cancer. Am J Epidemiol., 2005; 161: 153–157.
17. Hoffman F. Antioxidant vitamins newsletter. Nutr Rev., 1997; 14: 234–236.
18. Davì G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. Antioxid Redox Signal., 2005; 7: 256–268.
19. Hitchon C, El-Gabalawy H. Oxidation in rheumatoid arthritis. Arthritis Res Ther., 2004; 6: 265–278.
20. Cookson M, Shaw P. Oxidative stress and motor neuron disease. Brain Pathol., 2000; 9: 165–186.
21. Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. Neuropharmacology, 2001; 40: 959–975.
22. Lu Y.R., Yeap Foo L. Antioxidant activities of polyphenols from sage (*Salvia officinalis*) Food Chem., 2001; 75: 197–202.
23. Porto C.D., Calligaris S., Celloti E., Nicoli M.C. Antiradical properties of commercial cognacs assessed by the DPPH test. J. Agric. Food Chem., 2000; 48: 4241–4245.
24. Duan X., Wu G., Jiang Y. Evaluation of the antioxidant properties of litchi fruit phenolics in relation to pericarp browning prevention. Molecules, 2007; 12: 759–771.