

Phytochemical Composition and In-Vitro Antioxidant Activity of *Justicia* *WYNAADENSIS* Leaves

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ABSTRACT: Medicinal plants are primary source of healing and widely used for the treatment of various ailments. In the present study is to determine phytochemical analysis and antioxidant activity of *Justicia wynaadensis* family (Acanthaceae) by using different solvents such as petroleum ether, chloroform, ethylacetate, acetone, and methanol. The phytochemical analysis of all solvents crude extract showed positive result for alkaloids, flavonoids, glycosides, tannins terpenoids and steroids where as reducing sugar, saponins, anthroquinones, glycosides are absent. The In-vitro antioxidant activity of methanol extract from *Justicia wynaadensis* leaves determining by DPPH, reducing power, metal ion chelation, superoxide anion, nitric oxide, hydrogen peroxide and hydroxyl radical scavenging assay. The study showed that the methanol extract is a essential effect due to antioxidant activity.

KEYWORDS: *Justicia wynaadensis*, phytochemical screening, Antioxidant activity.

I. INTRODUCTION

The phytochemicals defined as "non-nutrient" chemicals found in plants that have biological activity against chronic diseases [1]. In the living system during the normal metabolic process, oxidation reactions produce unstable free radicals which start off chain reactions and damage the cell. While antioxidants are the free radical scavenging compounds and are able to terminate these chain reactions by being oxidized themselves and forming a stable radical [2,3]. These damages can be neutralized with natural antioxidants such as glutathione peroxidases, superoxide dismutases, catalase etc. and nutritional antioxidants from diet such as vitamins E, vitamins C and carotenoids etc [4]. In the present work, study of an Phytochemical and In-vitro antioxidant activity is carried out using methanol extract of *Justicia wynaadensis* leaves fig.1. The leaves is collected from Coorg district of South India[5]. The plant indicates the Medicinal value of leaves are sweetened and cooked with milk, and they are traditionally served during the monsoon, when it's supposed to be at the peak of its medicinal prowess [6].



Figure-1: Leaf of *Justicia wynaadensis*

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Vol. 4, Issue 9, September 2015

II. MATERIALS AND METHODS

The *Justicia wynaadensis* collected from Coorg district of South India. The collected leaves were washed thoroughly with tap water removal of soil particles. The leaves were shade dried and grind used for extraction [7].

Preparation of Plant Extract:

The *Justicia wynaadensis* of 100g leaves were first defatted using petroleum ether and were extracted using different solvents such as chloroform, acetone, ethyl acetate and methanol using Soxhlet apparatus. The extraction was carried out for 8 hours and the extract was thereafter concentrated by evaporation in rota-vacuum.

Phytochemical Analysis

Qualitative preliminary analysis of phytochemicals: Preliminary analysis of the phytochemicals were performed following the methods described given by [8,9,10].

Quantitative Estimation Of Phytochemicals

Estimation of total phenolics

Total phenolic content of methanol extract was measured using the method described given by [11].

Estimation of flavonoids

The flavonoid content of methanol extract was estimated using the method described given by [12].

In Vitro Antioxidant Assay

The antioxidant activity of methanol extract of *Justicia wynaadensis* leaves part were evaluated by the following methods.

DPPH Radical Scavenging Assay

Total antioxidant activity of plant extracts was estimated using stable DPPH radical scavenging assay method given by [13]. The inhibition of DPPH activity was calculated using equation 1.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of Test}) \times 100}{\text{Absorbance of Control}} \quad (1)$$

Metal ion Chelating Assay

The chelating potential of Fe^{2+} ion present in the extract was estimated by using 2,2'-bipyridyl competition method given by [14]. The inhibition of metal ion chelating activity was calculated using equation 1.

Superoxide Anion Radical Scavenging Assay

Superoxide anion radical scavenging activity was determined by NBT reduction method given by [15]. The inhibition of superoxide anion radical scavenging activity was calculated using equation 1.

Nitric Oxide Radical Scavenging Assay

Nitric oxide radical scavenging activity was determined by Griess reaction method given by [16]. The inhibition of nitric oxide radical scavenging activity was calculated using equation 1.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the methanol extract was determined using 2-deoxy-2-ribose oxidative degradation in Fe^{3+} -EDTA-Ascorbate- H_2O_2 system method given by [17]. The inhibition of hydroxyl radical scavenging activity was calculated using equation 1.

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging capabilities of the methanol extract of the plant part of leaves were determined as described given by [18].

III. RESULT AND DISSICUSSION

Qualitative Analysis:

Preliminary phytochemical screening indicate that the presence of alkaloids, flavonoids, glycosides, tannins and steroids in different solvent extracts of leaf of *Justicia wynaadensis* is shown in as is evident Table-1. Therefore methanol and ethyl acetate extract more phytochemical compare to other extracts.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 9, September 2015

Table -1. Indicate the results of qualitative analysis of Phytoconstituents of the different solvents extracts of *Justicia wynaadensis* species

Extract Test	Acetone	Petroleum Ether	Methanol	Ethyl Acetate	Chloroform
Fehling's	-	-	-	-	-
Frothing	-	+	-	-	-
Bortanger's	-	-	-	-	-
Flavonoids	-	+	+	+	-
Tannins	+	-	+	-	-
Alkaloids	-	+	+	+	+
Cardiac Glycosides	-	-	+	-	-
Terpenoids	-	+	-	+	-

“+” Present; “-” Absent.

Quantitative analysis:

Table 2: Indicate the results of quantitative estimation of Phenolics, Flavonoids, from methanol extract of *Justicia wynaadensis*.

<u>Fraction</u>	Total Phenolics content of leaves (mg GAE g ⁻¹ of extract)	Total Flavonoids content of Leaves (mg of QE g ⁻¹ of extract)
Methanol extract of <i>Justicia wynaadensis</i>	539.7	571.9

In vitro antioxidant assay of *Justicia wynaadensis* leaves of methanol extract

DPPH Assay

The antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH-H and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. This transformation results in a colour change from purple to yellow, which is measured spectrophotometrically, where in the disappearance of purple colour is monitored at 517 nm during the measurement of the absorbance [19]. The extract of the leaves demonstrates that it possesses a minimum inhibitory activity compared to ascorbic acid taken as reference standard as is evident from Fig.2. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a minimum potential activity of antioxidant property of the extract. IC₅₀ of Ascorbic acid was found to be 503.3979 µg/ml while that of the plant extract was found to be 540.2485µg/ml.

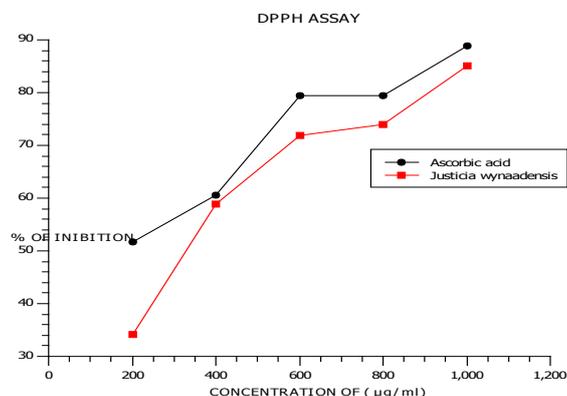


Fig-2. DPPH Radical Scavenging activities of Leaves part of *Justicia wynaadensis* and standard Ascorbic Acid. The graph shows that the extract has the almost same potency of total anti-oxidant activity as shown by the reference standard, suggesting its potential usage in scavenging activities.

Metal Ion Chelating Activity

The Ferrozine quantitatively chelates to Fe^{2+} ion producing a red colour complex. However, this reaction is limited in the presence of other chelating agents and results in a decrease in the intensity of the red colour of the ferrozine- Fe^{2+} complexes. A measurement of the colour reduction indicates that the chelating activity to compete with ferrozine for the Fe^{2+} ions [20]. The quantitative estimation of the colour reduction is measured spectrophotometrically at 523nm. The result revealed that extract of leaves has greater reduced metal ion chelating ability when compare to a reference standard EDTA as is evident from Fig.3. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a maximum potential activity of antioxidant property of the extract. IC_{50} of EDTA was found to be 1833.81 $\mu g/ml$ while that of the plant extract was found to be 1586.89 $\mu g/ml$.

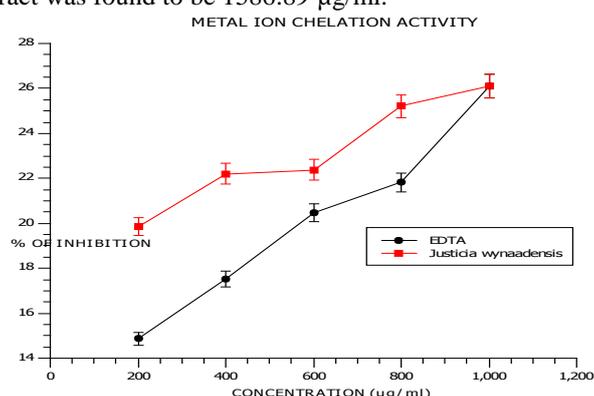


Fig-3. Metal Ion Chelating activities of Leaves part of *Justicia wynaadensis* and standard EDTA. The graph shows that the extract has the higher potency of metal chelation activity as compared to the reference standard, suggesting its potential usage in chelation activity.

Superoxide Radical Scavenging Activity

The superoxide anion a form of ROS which leads to tissue damage associated with inflammation in joint diseases such as rheumatoid arthritis, osteoarthritis, and crystal-induced arthropathies [21]. It was revealed that leaf extract has minimum reduced superoxide radical ability when compared to standard Ascorbic Acid as is evident from Fig.4. However, leaves extract also showed potent minimum reduced superoxide radical when compared to standard. IC_{50} of ascorbic acid was found to be 234.38 $\mu g/ml$ while that of the plant extract was found to be 162.16 $\mu g/ml$.

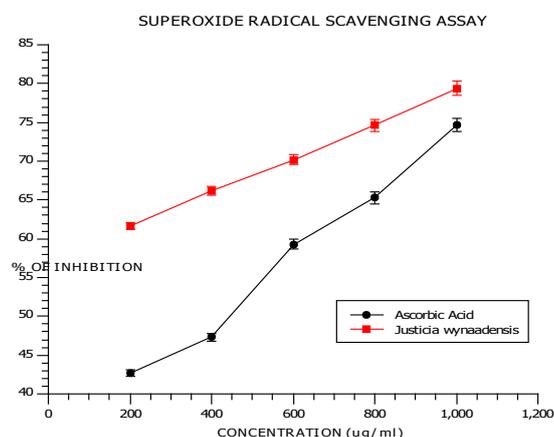


Fig-4. Superoxide radical scavenging activities of Leaves part of *Justicia wynaadensis* and standard Ascorbic Acid. The graph shows that the extract has the higher potency of radical scavenging activity as compared to the reference standard, suggesting its potential usage in neutralising the effects of free radicals that are generally produced during the diseased state like Diabetes mellitus.

Nitric Oxide Radical Scavenging Activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured using the Griess reaction reagent [22]. The result revealed that extract of leaves has minimum activity when compare to a reference standard Gallic acid as is evident from Fig.5. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a minimum potential activity of antioxidant property of the extract. IC₅₀ of gallic acid was found to be 641.5397 µg/ml while that of the plant extract was found to be 660.9385 µg/ml.

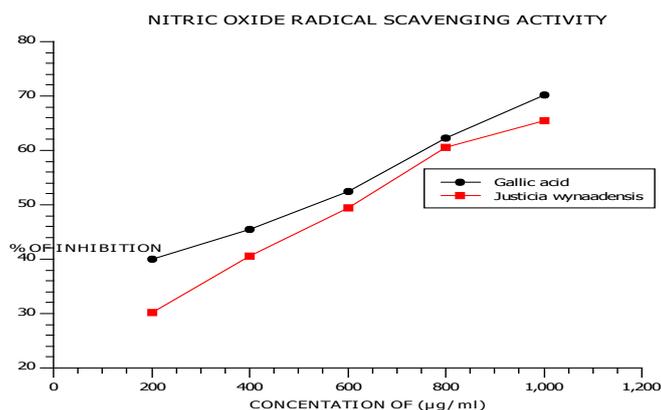


Fig-5. Nitric oxide radical scavenging activities of Leaves part of *Justicia wynaadensis* and standard Gallic acid.

The graph shows that the extract has the similar potency of radical scavenging activity as that of the reference standard, suggesting its potential usage in neutralising the effects of free radicals that are generally produced during the diseased state like Diabetes mellitus.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. It is widely used as a bleaching agent in the textile, paper and pulp industries. Hydrogen peroxide enters the human body through inhalation of vapour or mist and through eye or skin contact. In the body, H₂O₂ is

rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH[·]) that can initiate lipid peroxidation and cause DNA damage [23].The result revealed that extract of leaves has minimum activity when compare to a reference standard Ascorbic acid as is evident from Fig.6. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a minimum potential activity of antioxidant property of the extract. IC₅₀ of ascorbic acid was found to be 145.64 µg/ml while that of the plant extract was found to be 381.43 µg/ml.

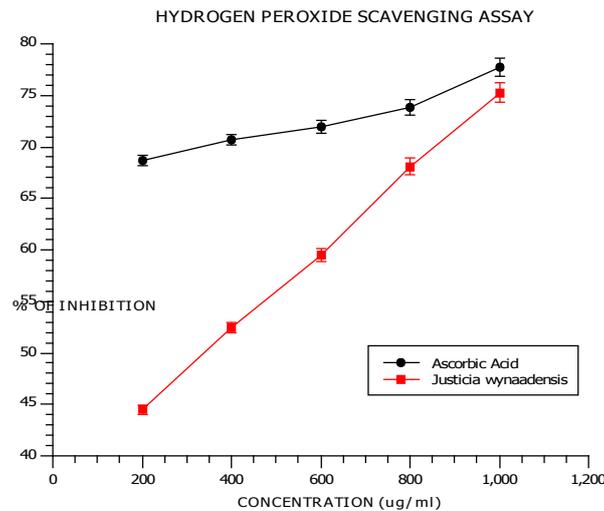


Fig-6. Hydrogen peroxide radical scavenging activities of Leaves part of *Justicia wynaadensis* and standard Ascorbic acid.

The graph shows that the extract has the relative lower potency of peroxide radical scavenging activity as compared to the reference standard, suggesting its limitations to use it as an antioxidants for peroxide radicals.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell [24]. The extract of the leaves demonstrates that it possesses a minimum inhibitory activity compared to mannitol taken as reference standard as is evident from Fig.7. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a minimum potential activity of antioxidant property of the extract. IC₅₀ of mannitol was found to be 1772.924 µg/ml while that of the plant extract was found to be 2746.197 µg/ml.

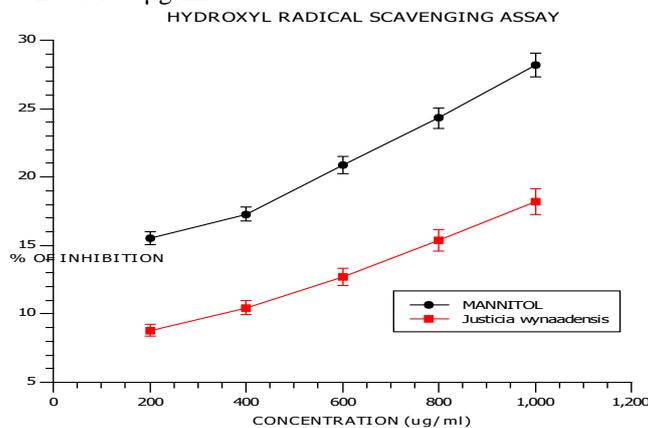


Fig-7. Hydroxyl radical scavenging activities of Leaves part of *Justicia wynaadensis* and standard Mannitol.

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(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 9, September 2015

The graph shows that the extract has the relative lower potency of hydroxyl radical scavenging activity as compared to the reference standard, suggesting its limitations to use it as an antioxidants for hydroxyl radicals.

Reducing power assay

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [25]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [26]. The extract of the leaves demonstrates that it possesses a minimum inhibitory activity compared to Ascorbic acid taken as reference standard as is evident from Fig.8. Therefore, the methanol extract of the leaves of *Justicia wynaadensis* indicates a minimum potential activity of antioxidant property of the extract. IC₅₀ of Ascorbic acid was found to be 336.9141 µg/ml while that of the plant extract was found to be 697.2514 µg/ml.

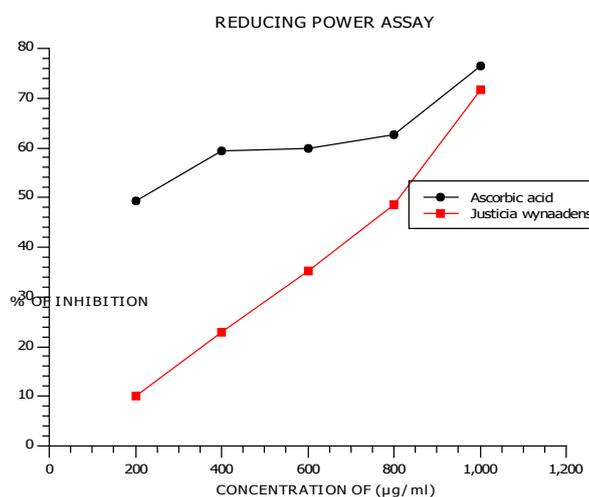


Fig-8. Reducing power activities of Leaves part of *Justicia wynaadensis* and standard Ascorbic acid.

The graph shows that the extract has the relative lower potency of reducing power activity as compared to the reference standard, suggesting its limitations to use it as reducing agents.

III. CONCLUSION

The Phytochemical analysis of *Justicia wynaadensis* from the above result methanol extract shown the presence of secondary metabolites such as alkaloids, flavonoids, alkaloids, tannins etc. The In-vitro antioxidant studies demonstrated that more inhibiting activity of superoxide, metal ion inhibition, hydroxyl radical scavenging activity. The DPPH, nitric oxide, hydrogen peroxide scavenging has a minimum antioxidant activity compare to a reference standard. Further analysis is required for HPLC, NMR compound isolation and identification.

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REFERENCES

1. Kushad, M., Masiuymas, J., Smith, M., Kalt, W., Eastman, k., "Health promoting phytochemicals in vegetables". Horticultural reviews ,Vol. 28, pp.125-185, 2003.
2. Kitazurua, ER., Moreirac, AVB., Mancini-Filhoc, J., Delinceed, H., Villavicencio, ALCH., "Effects of irradiation on natural antioxidants of Cinnamon (*Cinnamomum zeylanicum* N.)". Radiation Physics and Chemistry, Vol. 71, pp.37-39, 2004.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 9, September 2015

3. Aluyor, EO., Ori-Jesu, M., "The use of antioxidants in vegetable oils - A review". African Journal of Biotechnology, Vol.7(25), pp.4836-4842. 2008.
4. Zadák, Z., Hyspler, R., Tichá, A., Hronek, M., Fikrová, P., Rathouská, J., Hrnčiariková, D., Stetina, R., "Antioxidants and vitamins in clinical conditions". Physiol Res, Vol.58, pp.S13-7,2009.
5. <http://www.flowersofindia.net/catalog/slides/Wayanad%20Justicia.html>.
6. Chandra Mohan, S., balamurugan, V., "Antioxidant and phytochemical potential of medicinal plant kalanchoe pinnata". International journal of pharmaceutical sciences and research, Vol.3 (3),pp.881-885, 2012.
7. Abhishek M., Kirti Jain., Kanil Kumar, R.N., Somashekaraiah, B.V., "phytochemical Composition Of Psychotria *Dailzelli* Leaves". International Journal of Pharma and Bio Sciences. Vol.6(2), pp.688 – 692. 2015.
8. Harborne, JB., "Phytochemical Methods-A guide to modern techniques of plant analysis". Chapman and Hall, London. 1984.
9. Trease, GE., Evans, WC., "A Textbook of Pharmacognosy". Academic Press, London, .pp.22-40,1989.
10. Kokate, CK., Pharmacognosy. 16th edition, Nirali Prakashan, Mumbai, India.
11. Bray, HC., Thorpe, W., "Analysis of phenolic compounds of interest in metabolism". Methods of Biochemical Analysis, Vol.1, 27-52. 1954.
12. Chang, C., Yang, M., Wen, H., Chern, J., "Estimation of total flavonoid content in propolis by two complementary colorimetric methods" Journal of Food Drug Anal. Vol.10,pp.178-182, 2002.
13. Cotellet, A., Bernier, JL., Cateau, JP., Pommery, J., Wallet, JC., Gaydou, EM., "Antioxidant properties of hydroxyl flavones" Free Radic Biol Med, Vol.20,pp. 35-43,1996.
14. Yamaguchi, F., Ariga, T., Yoshimura, Y., Nakazawa, H., "Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind" Journal of Agric Food Chem, Vol.48,pp.180-185. 2000.
15. Nishimiki, M., Rao, NA., Yagi., "The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen" Biochemical and Biophysical Research Communications, Vol. 46, pp.849-853. 1972.
16. Green, LC., Wagner, DA., Glogowski, J., Skipper, PL., Wishnok, JS., Tannenbaum, SR., Analysis of nitrate, nitrite and (15N) nitrate in biological fluid. Anal Biochem, Vol.26,pp.131-138. 1982.
17. Kunchandy, E., Rao, MNA., "Oxygen radical scavenging activity of curcumin" International Journal of Pharmacol, Vol. 58,pp. 237-24, 1990.
18. Gulcin, I., Alici, HA., Cesur, M., "Determination of *in vitro* antioxidant and radical scavenging activities of propofol" Chemical and Pharmaceutical Bulletin, Vol.53,pp. 281-285, 2005.
19. Oktay, M., Gulein, v., Kufrevioglu, I., Labenson Wiss, U., "Technol", Vol36, pp.263-71,2003.
20. Soler Rivas, C., Espin, JC., Wichers, HJ., "An easy and fast test to compare total free radical scavenger capacity of foodstuffs". Phytochem. Anal. vol.11,pp.330-338, 2000.
21. Meyer, AS., Isaksen, A., "Application of enzymes as food antioxidants" Trends Food Sci. Tech. Vol.6,pp.300-304,1995.
22. Green, LC., Wagner, DA., Glogowski, J., Skipper, PL., Wishnok, JS., Tannenbaum, SR., "Analysis of nitrate, nitrite and 15N nitrate in biological fluids. Anal". Biochem. Vol.126,pp.131-138,1982.
23. Chanda, S., Dave, R., "In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties-An overview". African Journal of Microbiology Research. Vol. 3(13), pp. 981-996, 2009.
24. Halliwell, B., Gutteridge, JMC., "Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals" FEBS Lett. Vol.128, pp.347-352,1981.
25. Oktay, M., Gulcin, I., Kufrevioglu, OI., "Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts" Leb.-Wissen. Technol. Vol.36,pp.263-271. 2003.
26. Yen, GC., Chen, HY., "Antioxidant activity of various tea extracts in relation to their antimutagenicity" J. Agric. Food Chem. Vol.43,pp.27-32. 1995.