International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 11 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.11.006

Evaluation of *In vitro* Free Radical Scavenging potential of the Novel siddha formulation *Idivallathi Mezhugu* using DPPH, NO and ABTS radical scavenging assay

K.Nanthine[*1], S.Murugesan[2], R.Madhavan[3]

[*1]Post Graduate Scholar, Department of Nanju Noolum Maruthuva Neethi Noolum, National institute of Siddha, Tambaram Sanatorium, Chennai - 600047, Tamil Nadu, India

[2]Lectuer, Department of Nanju Noolum Maruthuva Neethi Noolum, National institute of Siddha, Tambaram Sanatorium, Chennai- 600047, Tamil Nadu, India

[3]Lecturer, Department of Nanju Noolum Maruthuva Neethi Noolum, National institute of Siddha, Tambaram Sanatorium, Chennai - 600047, Tamil Nadu, India

Corresponding Address: **Dr. K.Nanthine**, Post Graduate Scholar, Department of Nanju Noolum Maruthuva Neethi Noolum, National institute of Siddha, Tambaram Sanatorium, Chennai - 600047, Tamil Nadu, India.

E-mail: *nanthineksn@gmail.com*

Abstract

Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are products of normal cellular metabolism. The most common ROS include superoxide anion, H_2O_2, peroxyl (ROO-) radicals and reactive hydroxyl (OH-) radicals and the nitrogen derived free radicals are nitric oxide and peroxynitrite anion (ONOO-). These reactive species play an important role in pathogenesis of several oxidative stress related diseases like carcinogenesis, cardiovascular diseases, rheumatoid arthritis, ulcerative colitis and neurological degenerative diseases. India is known for its cultural, moral and also for traditional therapies. The widely practicing system among all is siddha system of medicine. Siddha is a novel therapy comprises of multidisciplinary approach it comprises of the herbal and polyherbal components. Often the formulation available in the Indian system of medicine is blend of herb and herbominerals by which it acts by multiple mechanism. One such novel formulation in siddha is *Idivallathi Mezhugu* (IM) used for treating various ailments in humans.There is an increasing trend to replace synthetic antioxidants, which are of safety concern with the natural antioxidants available from plant extracts or isolated products of plant origin. The main aim of the present investigation is to evaluate the antioxidant potential of the formulation IM using DPPH, NO and ABTS radical scavenging assay. The results of the study indicates that the DPPH radical scavenging activity of the formulation IM has shown dose dependent inhibition of radical ranges from 6.57 ± 5.57 to 51.49 ± 10.6 at the concentration varying from 10 to 100 µg/ml. NO radical scavenging activity of the IM has revealed potential radical scavenging activity ranges from 10.93 ± 1.59 to 57.81 ± 6.3. Similarly in ABTS assay the percentage inhibition ranges from 7.85 ± 2.28 to 61.33 ± 12.48 at the concentration varying from 10 to 100µg/ml. In conclusion the formulation IM possesses promising antioxidant activity against all three tested assay and hence it may be effective in the clinical management of the several oxidative stress related disorders.

Keywords: Siddha, *Idivallathi Mezhugu*, Antioxidant activity, Reactive Oxygen Species, DPPH, NO, ABTS.

1. Introduction

Free radicals have been directly implicated in various pathological conditions including diabetes mellitus, multiple sclerosis, heart disease, Parkinson's disease, inflammation, Alzheimer's disease, atherosclerosis, stroke, cancer, etc [1, 2]. Most of the body macromolecules, such as lipids, proteins, deoxyribonucleic acid [DNA] and carbohydrates are susceptible to damage by free radicals [3]. However, antioxidants have evolved with protective roles against such damage [4]. The negative cellular effects of ROS can be countered by enzymatic antioxidants such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutase (SOD), etc.; non-enzymatic, metabolic and nutrient antioxidants including glutathione, vitamin C, vitamin E, etc.; metal binding proteins like ferritin, lactoferrin, albumin, ceruloplasmin, etc., and phytochemicals such as quercetin, resveratrol, capsaicin etc [5].The mechanisms of protective actions of antioxidants against ROS toxicity include prevention of the formation of ROS, interruption of ROS attack, scavenging of the reactive metabolites or their conversion to stable molecules or molecules of lower reactivity [6].

Many medicinal plants, vegetables, and fruits have antioxidant components, especially phenolic compounds, which when consumed, have been confirmed to prevent the destructive/degenerative effects caused by oxidative stress [7]. Aside flavonoids and phenolic compounds which are widely distributed in plants; vitamin C, vitamin E, and carotenoids are some of the other antioxidant components of medicinal plants [8]. These phytoconstituents have been reported to exert various biological effects that include anti-inflammatory, free radical scavenging, anti-carcinogenic, anti-oxidant activities, etc [9]. Research activities focusing on medicinal plants have been encouraging because of their high content of potent antioxidants, accessibility, economic viability and next-to-no side effects [10].

Traditional medicine particularly herbal medicine playing important role in maintain of health in rural and remote areas. Inclusion of traditional herbal medicine in clinical practice will help to achieve the target ‘health for all’ Indian traditional medicine like Ayurveda and others have sound scientific background of effectiveness and also acknowledged by the recent researches. Although efforts are needed to overcome barriers like irrational use, quality control and standardization issues, high pharmacovogilance etc. Stick implementation of rules, monitoring and periodic revision of regulations are absolute necessary to promote Indian traditional medicine [11].

It is a well-known fact that Traditional Systems of medicines always played important role in meeting the global health care needs. They are continuing to do so at present and shall play major role in future also. The system of medicines which are considered to be Indian in origin or the systems of medicine, which have come to India from outside and got assimilated in to Indian culture are known as Indian Systems of Medicine [12].

Siddha system of medicine is believed as a brilliant achievement and symbol of Tamil culture which originated in Southern parts of India. Siddha medicine invented from Dravidian culture and is grown in the time of Indus valley civilization. Chinese alchemy, Taoism, and Taoist Patrology are considered as a main source of inspiration for Siddha alchemy. It is believed that in ancient time, the system was developed by eighteen siddhar (a class of Tamil sages). Though Siddha system of medicine resembles with Ayurveda in many aspects it has own philosophy and concept, holistic approach, and lifestyle oriented measures [13, 14, 15].

Even though siddha formulation are doing exceptionally good the documentary evidence of most of this indigenous preparations are highly limited and hence as a measure of the preparing the monograph for the novel siddha formulation *Idivallathi Mezhugu* (IM) the present study aimed at evaluating the antioxidant potential of the siddha formulation IM using DPPH, NO and ABTS radical scavenging assay.

2. Materials and Methods

2.1. DPPH (2, 2-Diphenyl 1-2 picrylhydrazyl) Assay [16]

The antioxidant activity of test drug was determined using the 2, 2-diphenyl 1-2 picrylhydrazyl (DPPH) free radical scavenging assay . Test drug IM was mixed with 95% methanol to prepare the stock solution in required concentration (10mg/100ml or 100µg/ml). 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 then10 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 sample

extract 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 sample extract at different concentration of (10 µg, 20 µg, 40 µg, 60 µg, 80 µg and 100 µg/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 sample 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.

2.2.Nitric Oxide Radical Scavenging Assay [17]

The concentrations of test sample 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% naphthylethylenediamine 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 extracts (10–100 µg/mL) and incubated at 25°C for 180 mins. The plant extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts 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 and standard was calculated and recorded. The percentage nitrite radical scavenging activity of the test drug IM and gallic acid were calculated using the following formula:

Percentage nitrite radical scavenging activity

$$\text{Nitic oxice scavenged (\%)} = \frac{A_{Control} - A_{Test}}{A_{Control}}$$

Where $A_{Control}$ = absorbance of control sample and A_{Test} = absorbance in the presence of the samples extracts of standards.

2.3. ABTS Assay [18]

This assay carried out for the purpose of evaluating the anti-oxidant potential of test drug IM 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 extract was measured following the same procedures described above and was used as positive controls. The antioxidant activity was calculated using the following equation: The ABTS scavenging effect was measured using the following formula:

Radical scavenging (%)

$$= \left[\frac{(A)_{Control} - (A)_{Sample}}{(A)_{Control}}\right] X100$$

3. Results

3.1. Effect of the formulation IM on DPPH radical scavenging activity

DPPH radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and ascorbic acid were used as standards. DPPH radical scavenging activity of the formulation IM has shown dose dependent inhibition of radical ranges from 6.57 ± 5.57 to 51.49 ± 10.6 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard ascorbic with percentage inhibition ranges from 32.19 ± 5.79 to 82.72 ± 2.19. IC 50 value of the formulation IM was found to be 100.8 ± 22.5 whereas for standard it was 37.24 ± 3.466. IM along with standard at all concentration showed a dose dependent inhibition on the DPPH radicals. As shown in table 1 and 2.

Table 1: Percentage inhibition of test drug IM on DPPH radical scavenging assay

Concentration (µg/ml)	% Inhibition of IM	% Inhibition of Ascorbic Acid
10 µg/ml	6.579 ± 5.57	32.19 ± 5.79
20 µg/ml	16.4 ± 5.29	44.47 ± 2.10
40 µg/ml	23.07 ± 7.00	52.54 ± 1.60
60 µg/ml	31.49 ± 7.16	60.96 ± 3.38
80 µg/ml	39.91 ± 7.00	71.49 ± 1.60
100 µg/ml	51.49 ± 10.6	82.72 ± 2.19

Data are given as Mean ± SD (n=3)

Table 2: IC50 Values for DPPH radical scavenging Assay by IM and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
ASCORBIC ACID	37.24 ± 3.466
IM	100.8 ± 22.5

Data are given as Mean ± SD (n=3)

3.2. Effect of the formulation IM on NO radical scavenging activity

NO radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and gallic acid were used as standards. NO radical scavenging activity of the formulation IM has revealed potential radical scavenging activity ranges from 10.93 ± 1.59 to 57.81 ± 6.3 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard gallic acid with percentage inhibition ranges from 31.42 ± 3.60 to 83.5 ± 2.08. IC 50 value of the formulation IM was found to be 86.21 ±9.71whereas for standard it was 37.36 ± 8.39. IM along with standard at all concentration showed a dose dependent inhibition on the NO radicals. As shown in table 3 and 4.

Table 3: Percentage inhibition of test drug IM on Nitric Oxide radical scavenging assay

Concentration (µg/ml)	% Inhibition of IM	% Inhibition of Gallic Acid
10 µg/ml	10.93 ± 1.59	31.42 ± 3.60
20 µg/ml	17.53 ± 2.16	42.18 ± 0.60
40 µg/ml	26.56 ± 3.00	52.25 ± 3.60
60 µg/ml	33.5 ± 3.60	65.1 ± 2.62
80 µg/ml	48.78 ± 4.21	73.78 ± 3.18
100 µg/ml	57.81 ± 6.36	83.5 ± 2.08

Data are given as Mean ± SD (n=3)

Table 4: IC50 Values for Nitric Oxide radical scavenging assay by IM and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
IM	86.21 ±9.71
GALLIC ACID	37.36 ± 8.39

Data are given as Mean ± SD (n=3)

3.3. Effect of the formulation IM on ABTS radical scavenging activity

ABTS radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and gallic acid were used as standards. ABTS radical scavenging activity of the formulation IM has shown significant radical scavenging activity ranges from 7.85 ± 2.28 to 61.33 ± 12.48 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard gallic acid with percentage inhibition ranges from 18.03 ± 8.28 to 73.21 ± 8.2. IC 50 value of the formulation IM was found to be 84.24 ±17.11 whereas for standard it was 56.92 ±10.88. IM along with standard at all concentration showed a dose dependent inhibition on the ABTS radicals. As shown in table 5 and 6.

Table 5: Percentage inhibition of test drug IM on ABTS radical scavenging assay

Concentration (µg/ml)	% Inhibition of IM	% Inhibition of Gallic Acid
10 µg/ml	7.85 ± 2.28	18.03 ± 8.28
20 µg/ml	18.11 ± 5.19	30.68 ± 5.26
40 µg/ml	27.26 ± 6.59	46.39 ± 6.73
60 µg/ml	40.82 ± 10.79	53.67 ± 3.04
80 µg/ml	45.95 ± 12.48	60.95 ± 10.62
100 µg/ml	61.33 ± 12.48	73.21 ± 8.28

Data are given as Mean ± SD (n=3)

Table 6: IC50 Values for ABTS radical scavenging assay by IM and standard.

Test Drug / Standard	IC50 Value DPPH Assay ± SD (µg /ml)
IM	84.24 ±17.11
GALLIC ACID	56.92 ±10.88

Data are given as Mean ± SD (n=3)

4. Discussion

Free radicals derived from oxygen, nitrogen and sulphur molecules in the biological system are highly active to react with other molecules due to their unpaired electrons. These radicals are important part of groups of molecules called reactive oxygen/ nitrogen species (ROS/RNS), which are produced during cellular metabolism and functional activities and have important roles in cell signalling, apoptosis, gene expression and ion transportation. However, excessive ROS attack bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, and cause oxidative stress, which can damage DNA, RNA, proteins and lipids resulting in an increased risk for cardiovascular disease, cancer, autism and other diseases. Intracellular antioxidant enzymes and intake of dietary antioxidants may help to maintain an adequate antioxidant status in the body. In the past decades, new molecular techniques, cell cultures and animal models have been established to study the effects and mechanisms of antioxidants on ROS. The chemical and molecular approaches have been used to study the mechanism and kinetics of antioxidants and to identify new potent antioxidants. Antioxidants can decrease the oxidative damage directly via reacting with free radicals or indirectly by inhibiting the activity or expression of free radical generating enzymes or enhancing the activity or expression of intracellular antioxidant enzymes [19].

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. When the stable DPPH radical accepts an electron from the antioxidant compound, the violet color of the DPPH radical was reduced to yellow colored Diphenylpicrylhydrazine radical which was measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers.

DPPH radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and ascorbic acid were used as standards. DPPH radical scavenging activity of the formulation IM has shown dose dependent inhibition of radical ranges from 6.57 ± 5.57 to 51.49 ± 10.6 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard ascorbic with percentage inhibition ranges from 32.19 ± 5.79 to 82.72 ± 2.19. IC 50 value of the formulation IM was found to be 100.8 ± 22.5 whereas for standard it was 37.24 ± 3.466. IM along with standard at all concentration showed a dose dependent inhibition on the DPPH radicals.

Nitric oxide is a potent pleiotropic mediator of various physiological processes. It is a diffusible free radical, which plays many roles as an effectors molecule in diverse biological systems [20]. No radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and gallic acid were used as standards. NO radical scavenging activity of the formulation IM has revealed potential radical scavenging activity ranges from 10.93 ± 1.59 to 57.81 ± 6.3 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard gallic acid with percentage inhibition ranges from 31.42 ± 3.60 to 83.5 ± 2.08. IC 50 value of the formulation IM was found to be 86.21 ±9.71whereas for standard it was 37.36 ± 8.39. IM along with standard at all concentration showed a dose dependent inhibition on the NO radicals.

ABTS radicals are more reactive than DPPH radicals, and the reactions with ABTS+ radicals involve a single-electron transfer process. Bleaching of a preformed solution of the blue-green radical cation ABTS+, which has absorption at 734 nm, has been extensively used to evaluate the antioxidant capacity of complex mixtures and individual compounds [21]. ABTS radical scavenging activity of the formulation IM at various concentrations ranging from 10 to 100µg/ml carried and gallic acid were used as standards. ABTS radical scavenging activity of the formulation IM has shown significant radical scavenging activity ranges from 7.85 ± 2.28 to 61.33 ± 12.48 at the concentration varying from 10 to 100 µg/ml when compare to that of the standard gallic acid with percentage inhibition ranges from 18.03 ± 8.28 to 73.21 ± 8.2. IC 50 value of the formulation IM was found to be 84.24 ±17.11 whereas for standard it was 56.92 ±10.88. IM along with standard at all concentration showed a dose dependent inhibition on the ABTS radicals.

5. Conclusion

From the results of the present investigation it was clear that the radical scavenging activity of the formulation IM were effective in the order ABTS > NO> DPPH. The maximum percentage inhibition were observed in ABTS assay with 61.33 followed by this the second highest inhibition observed in NO radical scavenging assay with 57.81 .DPPH radical assay ranked third with percentage inhibition of 51.49.The preventive and inhibitory effects of antioxidants on lipid peroxidation, DNA damage and protein modification caused by ROS are also discussed. The chemical approaches are simple and facilitate the study of the total antioxidant activity of antioxidants and the precise mechanisms of action of antioxidants. However, cell-free systems do not take bioavailability and metabolic factors into consideration, and thereby the data generated from these systems require the confirmation from cell-based systems or in vivo studies.

Acknowledgments

I wish to acknowledge my thanks to The Noble research solutions, Chennai, Tamil Nadu, India for their technical support for this research work.

6. References

1. Ozgen U, Mavi A, Terzi Z, Yildirim A, Coºkun M, Houghton PJ. Antioxidant properties of some medicinal Lamiaceae (Labiatae) species. Pharm Biol. 2006; 44: 107–112.
2. Tepe B, Sokmen M, Akpulat HA, Sokmen A. In vitro antioxidant activities of the methanol extracts of four Helichrysum species from Turkey. Food Chem. 2005; 90: 685–689.
3. Halliwell B, Gutteridge JM. Oxford: Clarendon Press. Free Radicals in Biology and Medicine, 1989: 96–98.
4. Jacob RA. The integrated antioxidant system. Nutr Res. 1995;15:755–66.
5. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables-the millennium's health. Int J Food Sci Tech. 2001; 36: 703–25.
6. Vinson JA, Su X, Zubik L, Bose P. Phenol antioxidant quantity and quality in foods: Fruits. J Agric Food Chem. 2001; 49:5315–5321.
7. Velioglu YS, Mazza G, Gao L, Oomah BD. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem. 1998; 46: 4113–4117.

8. Miller AL. Antioxidant flavonoids: Structure, function and clinical usage. Alt Med Rev. 1996; 1:103–111.
9. Auddy B, Ferreira M, Blasina F, Lafon L, Arredondo F, Dajas F, et al. Screening of antioxidant activity of three Indian medicinal plants, traditionally used for the management of neurodegenerative diseases. J Ethnopharmacol. 2003; 84:131–138.
10. Gbadegesin MA, Odunola OA. In vitro antioxidant/radical scavenging activities and hepatoprotective roles of ethanolic extract of Cassia occidentalis leaves in sodium arsenite-treated male Wistar rats. Br J Med Med Res. 2013; 3: 2141–2156.
11. Payyappallimana U. Role of traditional medicine in primary health care: an overview of perspectives and challenges. Yokohama J Soc Sci. 2010;14:57–77
12. Prasad LV. In: Indian System of Medicine and Homoeopathy Traditional Medicine in Asia. ChaudhuryRanjit Roy, RafeiUtonMuchatar., editors. New Delhi: WHO- Regional Office for South East Asia. 2002: 283–286.
13. Subbarayappa BV. The roots of ancient medicine: an historical outline. J Biosci. 2001; 26:135–144.
14. Karunamoorthi K, Jegajeevanram K, Xavier J.Tamil traditional medicinal system – siddha: an indigenous health practice in the international perspectives. Tang. 2012; 2:1–11.
15. Subbarayappa B.V. Siddha medicine: an overview. Lancet. 1997; 350:1841–1844.
16. Badami, Omprakash, DongrSH,Suresh B. In-vitro Antioxidant property of SolanumPseudocapsicum leaf extract. Indian J Pharmacol.2005; 37:251-252.
17. B. N. Panda, A. B. Raj, N. R. Shrivastava, and A. R. Prathani, “The evaluation of nitric oxide scavenging activity of Acalyphaindica Linn Root,” Asian Journal Research Chemistry.2009; 2:148–150.
18. 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 cationdecolorization assay. Methods in Enzymology .1999; 299:384–389.
19. Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM .Antioxidant activity of methanol extract of Ferula assafoetida and its essential oil composition. GrasasAceites .2009;60: 405-412.
20. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA. Amelioration of chronic ileitis by nitric oxide synthase inhibition. J PharmacolExpTher .1993; 264: 11-16.
21. Jian-Ming Lu, Peter H Lin.Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems.J Cell Mol Med. 2010; 14: 840–860.

How to cite this article:
K.Nanthine, S.Murugesan, R.Madhavan. (2017). Evaluation of *In vitro* Free Radical Scavenging potential of the Novel siddha formulation *Idivallathi Mezhugu* using DPPH, NO and ABTS radical scavenging assay. Int. J. Adv. Res. Biol. Sci. 4(11): 42-48.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.11.006