DOI: 10.55522/jmpas.v3i2.0044 ISSN NO. 2320 – 7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Review article

A review on in vivo and in vitro methods used for screening antioxidant activity

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Received – 20 February 2014**, Revised** - 25 March 2014, **Accepted** – 23 April 2014 **(DD-MM-YYYY)**

Refer This Article

Manpreet Kaur, Satwinder Kaur, Amandeep Kaur, 2014. A review on in vivo and in vitro methods used for screening antioxidant activity. Journal of medical pharmaceutical and allied sciences, V 3 - I 2, Pages -170– 172. Doi: https://doi.org/10.55522/jmpas.V3I2.0044.

ABSTRACT

Antioxidant activity has been assessed in many ways. The combination of all approaches with the many test methods available explains the large variety of ways in which results of antioxidant testing are reported. Several test procedures may be required to evaluate such antioxidant activities. The methods are broadly classified as in vitro and in vivo methods. And those are described and discussed below in this review article. As per this review there are 6 in vitro methods and 5in vivo methods that are being used for the evaluation of antioxidant activity of the sample of interest. DPPH method was found to be used mostly for the in vitro antioxidant activity evaluation purpose while LPO was found as mostly used in vivo antioxidant assay.

Keywords: Moringa oleifera, melia azederach, rubia cardifolia, flavonoids, diuretic antilithic, Terpinoides.

INTRODUCTION

Metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes. Any free radical involving oxygen is then referred to as reactive oxygen species (ROS). The most commonly formed ROS are superoxide anion radical (O2•−) and hydroxyl radical (•OH). Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis. Protection against free radicals can be enhanced by ample intake of dietary antioxidants.Various methods are used to investigate the antioxidant property of samples of diets, plant extracts, commercial antioxidants etc. The objective of this review article is to accumulate all probable methods that are used to evaluate the antioxidant property of various samples. This review can reduce the time for literature review for scientific research. A ompiled description of all available in vitro and in vivo antioxidant models are described in this review which are most commonly used in small scale laboratories.[1, 2].

METHODS USED FOR SCREENING OF ANTIOXIDANT ACTIVITY *IN VITRO* METHODS

Generally *in vitro* antioxidant tests using free radical traps are relatively straightforward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models.

DPPH scavenging activity

According to Brand-Williams et al the antioxidant activity was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical by plant extract. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of sample solution of varying concentrations to be tested. Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV- Vis spectrophotometer. The inhibition % was calculated using the following formula.

Inhibition % = Ac-As/Ac×100

Ac is the absorbance of the control As is the absorbance of the sample

Hydrogen peroxide scavenging (H_2O_2) assay

According to Ruch et al. the ability of plant extracts to scavenge hydrogen peroxide can be estimated by following method. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 m M pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. Extract (20–60 μg/mL) in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was measured spectrophotometrically. Plant Extract prepared in ethanol was added to different test-tubes in varying concentrations. Sodium nitroprusside (5mM) in phosphate buffer was added to each test tube to make volume up to 1.5ml. Solutions were incubated at 25ºC for 30 minutes. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylenedi amine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

Superoxide radical scavenging activity (SOD)

Nishikimi et al. described the method for evaluating antioxidant

activity. The reaction mixture consisted of 1ml of NBT solution (156μM) and sample solution at different concentrations. The reaction was started by adding 100μl of phenazin methosulfate solution (60μM, PMS) in phosphate buffer (pH7.4) to the reaction mixture followed by incubation at 25oC for 5 min and the absorbance at 560 mm was measured against blank. Ascorbic acid was used as the standard. Superoxide scaven ging activity (%) = Abs control-Abs sample/Abs control ×100

Abs control: Absorbance of the control and Abs test: Absorbance of the extracts/standar d.

Hydroxyl radical scavenging activity

The scavenging ability of hydroxyl radicals is measured by the method of Kunchandy and Rao. Hydroxyl radical is one of the potent reactive oxygen species in the biological system that reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cellThe reaction mixture (1.0 mL) consist of 100 μL of 2-deoxy-Dribose (28 mM in 20 mM KH2PO4-KOH buffer, pH 7.4), 500 μL of the extract, 200 μL EDTA (1.04 mM) and 200 μM FeCl3 (1:1 v/v), 100 μL of H2O2 (1.0 mM) and 100 μL ascorbic acid (1.0 mM) which is incubated at 37 °C for 1 h. One milliliter of thiobarbituric acid (1%) and 1.0 mL of trichloroacetic acid (2.8%) are added and incubated at 100 °C for 20 min. After cooling, absorbance is measured at 532 nm, against a blank sample.

Thiobarbituric acid (TBA) method

According to Ottolenghi (1959) the method isis as follows: The final sample concentration of 0.02% w/v was used in this method. Two mL of 20% trichloroacetic acid and 2 mL of 0.67% of thiobarbituric acid were added to 1 mL of sample solution. The mixture was placed in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum.

In Vivo Models

For all in vivo methods the samples that are to be tested are usually administered to the testing animals (mice, rats, etc.) at a definite dosage regimen as described by the respective method.After a specified period of time, the animals are usually sacrificed and blood or tissues are used for the assay.

Reduced glutathione (GSH) estimation

According toEllman (1959) antioxidant activity can be determined by following method. The tissue homogenate (in 0.1 M phosphate buffer pH 7.4) is taken and added with equal volume of 20% trichloroacetic acid (TCA) containing 1 mM EDTA to precipitate the tissue proteins. The mixture is allowed to stand for 5 min prior to centrifugation for 10 min at 2000 rpm [3, 4].

The supernatant (200 μL) is then transferred to a new set of test tubes and added with 1.8 mL of the Ellman's reagent (5,5′-dithiobis-2- nitrobenzoic acid (0.1 mM) prepared in 0.3 M phosphate buffer with 1% of sodium citrate solution). Then all the test tubes are made up to the volume of 2 mL. After completion of the total reaction, solutions are measured at 412 nm against blank. Absorbance values were compared with a standard curve generated from known GSH.

Glutathione peroxidase (GSHPx) estimation

According to Wood (1970), Cytosolic GPx is assayed via a 3-mL cuvette containing 2.0 mL of 75 mM/L phosphate buffer, pH 7.0. The following solutions are then added: 50 μL of 60 mM/L glutathione reductase solution (30 U/mL), 50 μL of 0.12 M/L NaN3, 0.10 of 0.15 mM/L Na2EDTA, 100 μL of 3.0 mM/L NADPH, and 100 μL of cytosolic fraction obtained after centrifugation at 20,000 g for 25 min. Water is added to make a total volume of 2.9 mL. The reaction is started by theaddition of 100 μL of 7.5 mM/L H2O2, and GSHPx was expressed in terms of mg of proteins.

Superoxide dismutase (SOD) method

According to Mccord and Fridovich (1969) the antioxidant activity of a sample is estimated in the erythrocyte lysate prepared from the 5% RBC suspension. To 50 μL of the lysate, 75 mM of Tris–HCl buffer (pH 8.2), 30 mM EDTA and 2 mM of pyrogallol are added. An increase in absorbance is recorded at 420 nm for 3 min by spectrophotometer. One unit of enzyme activity is 50% inhibition of the rate of autooxidation of pyrogallol as determined by change in absorbance/min at 420 nm. The activity of SOD is expressed as

units/mg protein.

Catalase (CAT)

According to Aebififty microliter of the lysate is added to a cuvette containing 2 mL of phosphate buffer (pH 7.0) and 1 mL of 30 mM H2O2. Catalase activity is measured at 240 nm for 1 min using spectrophotometer. The molar extinction coefficient of H O , 43.6 M cm−1 was used 2 2 the conversion of NADPH to NADP is monitored by a continuous recording of the change of absorbance at 340 nm at 1 min interval for 5 min. Enzyme activity of to determine the catalase activity. One unit of activity is equal to 1 mmol of H2O2 degraded per minute and is expressed as units per milligram of protein.

Lipid peroxidation (LPO) assay

According to Ohkawa et al. (1979) the tissues are homogenized in 0.1 M buffer pH 7.4 with a Teflon-glass homogenizer. LPO in this homogenate is determined by measuring the amounts of malondialdehyde (MDA) produced primarily. Tissue homogenate (0.2 mL), 0.2 mL of 8.1% sodium dodecyl sulfate (SDS), 1.5 mL of 20% acetic acid and 1.5 mL of 8% TBA are added. The volume of the mixture is made up to 4 mL with distilled water and then heated at 95 °C on a water bath for 60 min using glass balls as condenser. After incubation the tubes are cooled to room temperature and final volume was made to 5 mL in each tube. Five mL of butanol: pyridine (15:1) mixture is added and the contents are vortexed thoroughly for 2 min. After centrifugation at 3000 rpm for 10 min, the upper organic layer is taken and its OD is taken at 532 nm against an appropriate blank without the sample. The levels of lipid peroxides can be expressed as n moles of thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of 1.56×10^5 ML cm^{-1} [11-14].

CONCLUSION

This review article is focused on in vitro and in vivo methods of antioxidant evaluation. Presently, 6 in vitro and 5 in vivo methods are being used for antioxidant evaluation purpose. DPPH method is the most frequently used one for in vitro antioxidant activity evaluation while LPO was found as the mostly used in vivo antioxidant assay. This article will be a comprehensive ready reference for those who are interested on antioxidant study.

REFERENCES

1. Heath D. Scheibmeira, Katie Christensena, 2005. A review of free radicals and antioxidants for critical care nurses. Intensive and Critical Care Nursing. 21, Pages 24—28.

2. Kinnula VL, Crapo JD, 2004. Superoxide dismutases in malignant cells and human tumors, Free Radic. Biol. Med, 36, Pages 718–744.

3. Coasta P, Manuel J, Labao S. 2002.Modelling and comparison of dissolution profiles. Euro. J. Pharma. Sci. 13, Pages 123-133.

4. Sune B, Folke E, Liisa TK, Maria R, 1995. Selective enzymatic reactions using Micro emulsion-based gels. Colloidal and Surface B. Biointe. 4, Pages 121-127.

5. Joshi B, 2011. Emulgel: A Comprehensive Review on the Recent Advances in Topical Drug Delivery. International Research Journal of Pharmacy. 2(11), Pages 66-70.

6. Zhu W et al, 2009. Micro emulsion-based hydrogel formulation of pencclovir for topical delivery. International Journal of Pharmaceutics. 378, Pages 152-158. Doi: 10.1016/j.ijpharm.2009.05.019.

7. Sevgi G, Sedef Erdal M, Buket A, 2013. New Formulation Stratagies in Topical Antifungal Therapy. Journal of Cosmetics, Dermatological Sciences and Application. 3, Pages 6-65. Doi: 10.4236/jcdsa.2013.31A009.

8. C Chandran S, Dr Shirwaikar A, Dr Drminic, 2011. Development and Evaluations of Ethosomal Formulation containing Ketoconazole. Asian journal of Biomedical and Pharmaceutical Research. 1(4), Pages 303-309.

9. Dadwal M, 2013. Emulgel: A Novel Approach to Topical Drug Delivery. International Journal of Pharma and Bio Sciences. 4(1), Pages 847-856.

10. Mehta K, Bhatt DC, 2011. Preparation, Optimization and In Vitro Microbiological Efficacy of Antifungal Microemulsion. International Journal of Pharmaceutical Sciences and Research. 2(9), Pages 2424-2429.

11. Kaur LP, Guleri TK, 2013. Topical gel: A Recent Approach for Novel Drug Delivery. Asian Journal of Biomedical & Pharmaceutical Sciences. 3(17), Pages 1-5. Doi: 10.15272/AJBPS.V3I17.183.

12. Guidi I, Galimberti D, Lonati S, 2006. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol, Aging. 27, Pages 262–269.

13. Bolton JL, Trush MA, Penning TM, 2000. Role of quinones in toxicology. Chem. Res Toxicol 13, Pages 135–160.