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FREE RADICAL SCAVENGING ACTIVITY OF HISPOLON AND HISPOLON DERIVATIVE: AN IN VITRO STUDY

Prasanth D, Swathi P, Eswar Kumar K,

A.U. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam - 530003

Associate Professor,A.U.College of Pharmaceutical Sciences,Andhra University, Visakhapatnam – 530003

ABSTRACT:

The present study was aimed to evaluate the free radical scavenging activity of hispolon and hispolon mono methyl ether (HMME) using different model systems such as nitric oxide and DPPH free radical scavenging activities; Ferric reducing antioxidant power (FRAP) and ABTS radical scavenging activity. Results of the study indicated that the inhibitory concentrations (IC 50) of hispolon (25.66), HMEE (24.84) and ascorbic acid (34.10)in nitric oxide free radical scavenging activity and hispolon (13.10), HMEE (26.92) and ascorbic acid (22.21) were observed in DPPH radical scavenging activity. The FRAP and TEAC values were also found to be significantly increased with hispolon and HMME in a concentration dependent manner compared with ascorbic acid. The antioxidant activity of hispolon and HMME might be due to electro donating capacity or hydrogen donating capacity to scavenge DPPH, nitric oxide and ferric ions.

KEYWORDS: Hispolon, FRAP, TEAC, Free radicals

INTRODUCTION:

Free radicals are being one of the important causes of many chronic disease conditions. Which are also been formed due to chemotherapy, radiation, and environment pollutants and also during consequences in neurodegenerative conditions such as ageing, parkinson's disease and also in cerebral ischemia (Pavithra K., 2015). The free radical means any species which contain one or more unpaired electrons; they may interfere in regular function and reactivity of an atom/ molecule and make them more reactive than regular. The free radicals categorized into reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated from either endogenous or exogenous sources. Endogenous free radicals are generated from immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, aging (Lien Ai PH., 2008). The source of exogenous free radicals are from air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, tacrolimus, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil, fat), radiation.

The condition when oxidants overwhelm the antioxidant protective system called as oxidative stress. The imbalance between free radicals (ROS & RNS) and enzymatic (superoxide

dismutase, glutathione peroxidase, and catalase) and non enzymatic antioxidants (glutathione, vitamin C, E and D) may leads to oxidative stress (Loperena, R., 2017). The primary oxygen free radical generated by NADPH oxidase during mitochondrial respiratory electron transport chain. Further the proceeded to hydrogen peroxide (H_2O_2); excess H_2O_2 forms hydroxyl ions (HO^-) through metal catalyzed reaction and finally targets the carbohydrates, protein, nucleic acid and make them malfunctioned by altering their structure and functions (Reuter, S., 2010).

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention.Natural products gained attention either from plant or fungal sources in leading drug discovery because of their safety, efficacy and selectivity. Among, polyphenols are diverse group of compounds and proven medicinal as well as in industrial potentiality (Pandey KV., 2009). Ascorbic acid at 10 folds of higher concentrations even did not produce similar actions like polyphenols (Grace, M.H., 2014). The potential actions of polyphenols in chronic diseases by attenuating the inducible nitric oxide synthase activity, inhibiting lipid peroxidation, decreasing the number of immobilized leukocytes during reperfusion and finally controlling the inflammatory responses (Jens L., 2010).

Consumptions of mushrooms are increased now a day due to wide therapeutic actions; Phenolic compounds appear to be the main components responsible for antioxidant activity of extracts from mushrooms. A total of 28 phenolic compounds were detected in the mushrooms studied. The average total concentration of phenolic compounds are 326 microg/g, the average being of 174 microg/g in edible mushrooms and 477 microg/g in medicinal mushrooms (Naznoosh S., 2019).Hispolon is a polyphenolic compound derived from black hoof mushroom (Phellinuslinteus) or shaggy bracket mushroom (Inonotushispidus), inorder to identify the role of hispolon in antioxidant potential of mushroom, the present study is aimed to investigate the antioxidant activity of hispolon and hispolonmonomethyl ether derivative (HMME) using

1. Nitric oxide radical scavenging activity
2. Ferric reducing antioxidant power (FRAP)
3. $ABTS^+$ radical scavenging activity
4. DPPH radical scavenging activity

MATERIALS AND METHODS:

Chemicals: Hispolon and hispolonemonomethyl ether (HMME) are obtained as gift samples from pharmaceutical chemistry department, A.U.College of pharmaceutical sciences, Andhra University. Remaining all the chemicals are obtained from Sisco research laboratories, Mumbai.

Ferric Reducing Ability Power (FRAP)

The antioxidant ability of serum was estimated by using capability to reduce Fe3 + to Fe2 + by a FRAP (Ferric Reducing Ability of Plasma) test. The FRAP method estimates a absorption change at 593 nm due to the formation of the color Fe (II) -tripyridyltriazine blue from Fe (III) by the action of donating antioxidants. Te FRAP reagent comprised with acetate buffer (300mmol/L) and acetate buffer with pH 3.6, also contains TPTZ (10mmol), FeCl3 (20mmol) and water in ratio of 10:1:1. To 10mL of serum was added to pre warmed FRAP reagent at 37^0C

in a test tube and incubated for 10min at 37^0C. The absorption of the blue-colored complex was studied compared to the blank substance (300 mL FRAP reagent + 10 mL water distilled) at 593 nm. Typical Fe2 + solutions per 100 to 1000 mmol are prepared from the ferrous sulphate (FeSO47 H2O) in water. The data was presented as mmol ferric ions reduced to ferrous form for liter (FRAP value) (Benzie, 1996).

ABTS radical scavenging assay:

ABTS radical cation depolarization assay was aimed to identify the ability of serum antioxidant capacity (Re R., 1999). ABTS • + radical was developed to react between 7 mM ABTS in water and 2.45 mM potassium persulfate (1: 1) and prior to use need to store in the dark at room temperature for 12-16 h. After that the prepared solution was diluted with methanol to obtain required absorbance 0.700 at 734nm. Once after adjustment the 5 µl of serum sample was added to 3.995 ml of diluted ABTS·+ solution. The final mixture absorbance was measured after 30min of the preparation. The blanking was conducted using the suitable solvent. All measurements were made at least three times. All the measurements were carried out at least three times. Percent inhibition of absorbance at 734 nm was calculated using the formula, Percentage inhibition at 734 nm were calculated using a formula, ABTS • + radical scavenging effect (%) = ((AB-AA) / AB) × 100 (2), whereas, AB absorption of ABTS radical + methanol; AA serum / standard ABTS sample. Trolox was used as a standard.

DPPH radical scavenging activity:

The potential AA of extracts, fractions and pure compounds was determined on the basis of the scavenging activity of the stable 1,1 -diphenyl-2-picrylhydrazyl (DPPH) free radical. Aliquots of 1ml of a methanolic solution containing each pure compound were added to 3ml of 0.004% MeOH solution of DPPH. Absorbance at 517 nm, against a blank of methanol without DPPH, was determined after 30 min (UV, Perkin-Elmer-Lambda 11 spectrophotometer)and the percent inhibition activity was calculated. IC50 values denote the concentration of samplerequired to scavenge 50% DPPH free radicals. All testswere run in triplicate and averaged (Braca A., 2001).

Nitric oxide radical scavenging activity:

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and IBPG and the reference compound in different concentrations (20, 40, 60, 80 and 100 µg) were incubated at 25°C for 150 min. After incubation 1.5ml of the Griess reagent (1% sulphanilamide, 0.1% naphthyl ethylene diaminedihydrochloride in 2% H3PO4) was added. The absorbance of the chromophore formed was measured at 546nm. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test samples (Ebrahimzadeh MA., 2010).

DISCUSSION:

In humans, oxidation is a process that the body uses for normal energy production and immune function. This is part of the process that enables the body to transform nutrients such as carbohydrates, fats, and proteins into energy. During oxidation, ROS are produced at low levels in normal physiological conditions, which are necessary for maintaining normal cell functions, and the endogenous antioxidant defense systems of the body have the capacity to avert any

harmful effects.Recently, exploration of natural sources as novel antioxidant bioactive compounds has gained considerable attention and it has helped to provide therapeutic drugs and principal compounds. Mushrooms, traditionally known as a valuable source of natural bioactive compounds, have been studied widely for their therapeutic capabilities(Kozarski M., 2015).Plant polyphenols are secondary metabolites characterized by one or more hydroxyl groups binding to one or more aromatic rings (Zhou, Y., 2019). Several thousand polyphenolic molecules have been identified in higher plants, including edible ones. A number of studies have shown that plant polyphenols can be used as antioxidants against different oxidative stress-induced diseases (Boo, Y.C., 2019). Based on importance of polyphenols in control over free radicals in diseases, the present study was planned to conduct free radical scavenging activity of mushrooms derived polyphenol hispolon and HMME using various in vitro radical scavenging activity assays.

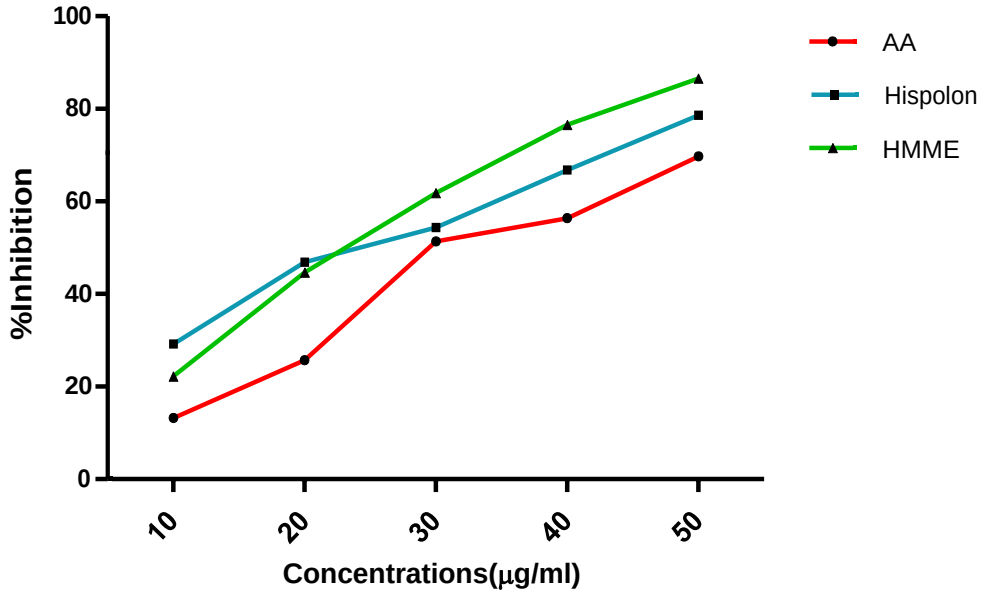
A series on events occur in the generation of free radicals form initial stage to final stage of oxidative reactions either from metabolic events or from exogenous source. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. Nitric oxide (NO) is generated from amino acid L-arginine by vascular endothelial cells, phagocytes, and certain cells of the brain. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion ($ONOO^-$) (Nagmoti DM., 2001).The present study both hispolon and HMME also showed better scavenging activity of nitric oxide radical in dose dependent manner as shown in figure 1. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The nitrite ions diazotize with sulphanilamide acid and couple with naphthylethylenediamine, forming pink colour, which was measured at 546 nm.The decreased absorbance withhispolon and HMMEdue to donationof protons to the nitrite radical thereby decreases in absorbance indicating the extent of nitrite radical scavenging (Turkoglu A., 2007).

The ferric reducing antioxidant power (FRAP) assay is measures the reduction of ferric ion (Fe^{3+})-ligand complex to the intensely blue-colored ferrous (Fe^{2+}) complex by antioxidants in an acidic medium to drive electron transfer in order to maintain iron solubility.In the present study both hispolon and HMME also showed similar activity of ferric reducing antioxidant activity by reducing the ferric form to ferrous form by donating one electron compared with ascorbic acid (figure 2). To assess the anti radical activity of the hispolon and HMME, the study extended to measure trolox equivalent antioxidant capacity. The assay determines the antioxidant activity against scavenging stable radical cation ABTS+ (2,2′-azinobis(3- ethylbenzothiazoline-6-sulfonic acid)),a blue-green chromophore with maximum absorption at 734 nmin terms of TEAC values. Antioxidant compounds with electron donation or hydrogen atom donation capability can quench the $ABTS^+$ radical (Prior et al., 2005). Both hispolon and HMME showed marked antioxidant activity due to electron donating to $ABTS^+$ radical(figure 3). The hispolon and HMME was able to reduce the stable DPPH radical to diphenylpicryl hydrazine, a yellow color compound due to hydrogen donating capacity in order to remove the odd electron of DPPH which will be responsible for free radical activity (figure 4).

The nitric oxide and DPPH radical scavenging activity and FRAP and TEAC potential might be due to polyphenolic nature of hispolon and hispolon mono methyl ether are mainly due to the redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers, in addition to their metal chelating potential. The antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals (Turkoglu A., 2007).Many studies were conducted previously for antioxidant activity of individual edible mushrooms and other polyphenols of mushrooms. Example: Phellinuslinteuswas reported for

TEAC activity and DPPH radical scavenging activity due to the presence of hispidin (Jung JY., 2008). Heng-Yuan C., 2007 reported about antioxidant activity of Phellinusmerrillii with highest TEAC, FRAP and DPPH radical scavenging activity. Isoxazole and pyrazole derivatives of hispolon was also tested for free radical scavenging activity, both are showed good DPPH radical scavenging activity (Shaukat Ali MS., 2016).

The study concluded that the both hispolon and hispolonmonomethyl ether (HMEE) showed superior activity than ascorbic acid, among HMEE produced better activity compared with hispolon alone might be due to the methyl etherfunctional group.Many reported antioxidants with methyl ether showed potent antioxidant activity such asnaringenin-4',7-dimethyl-ether and Isoliquiritigenin-4-methyl-ether (Fernanda RG., 2006) for antioxidant and cyoprotective activities; Quercetinmonomethyl ethers such as azaleatin, isorhamnetin, tamarixetin showed significant radical scavenging activity (Pandurangan N., 2019);Hydroquinone mono methyl ether (HMME) is industrially a very important anti-oxidant (Ganapati D., 2005); ellagic acid monomethylether(El Raey, M., 2014) reported for antioxidant, gastroprotective and hepatoprotectiveactivites. It is concludedthat hispolon and hispolon monomethyl ether produced antioxidant activity more significantly than ascorbic acid might be due to the polyphenolic nature and due to its functional group monomethylether of hispolon.



IC IC50: AA: 34.10; Hispolon:25.66; HMEE:24.84

Figure 1: Effect of ascorbic acid, hispolon and HMME on nitric oxide radical scavenging activity

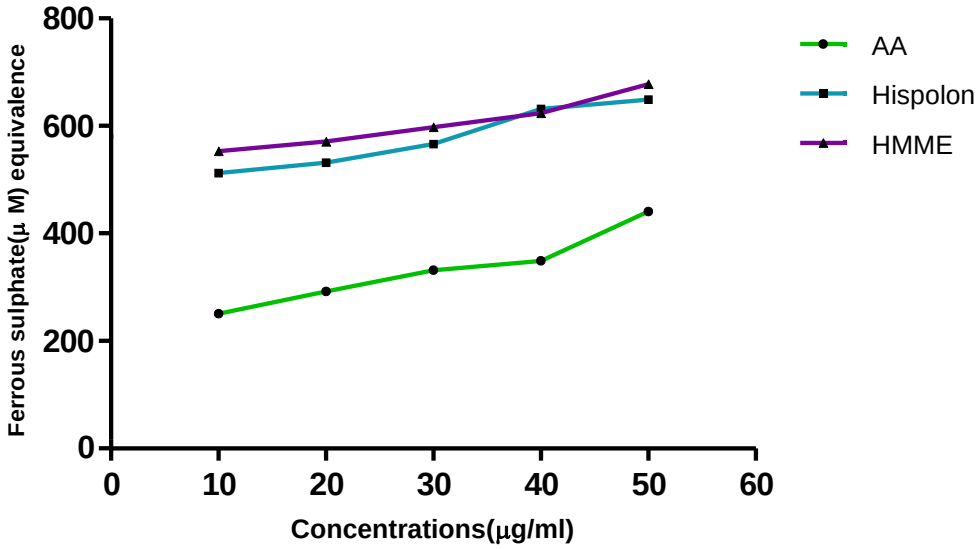


Figure 2: Effect of ascorbic acid, hispolon and HMME on Ferric reducing antioxidant power

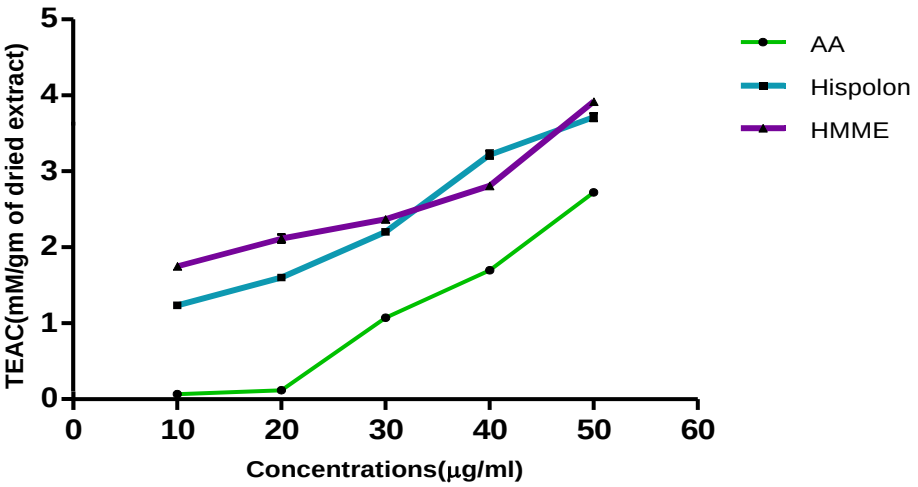
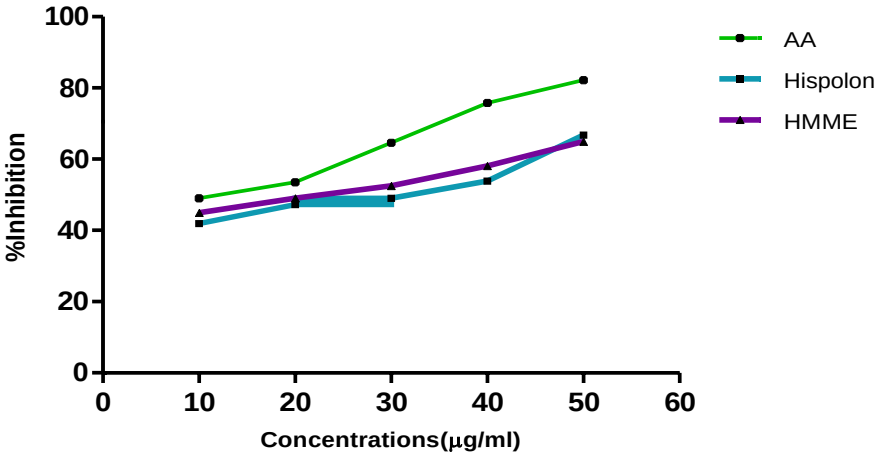


Figure 3: Effect of ascorbic acid, hispolon and HMME on $ABTS^{+}$ radical scavenging activity



IC50: AA: 22.21; Hispolon: 13.10; HMEE: 26.92

Figure 4: Effect of ascorbic acid, hispolon and HMME on DPPH radical scavenging activity

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