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EVALUATION OF ANTIOXIDANT ACTIVITY IN POLYHERBAL EXTRACT

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ABSTRACT

The reaction of oxidation is universally important reaction taking place in living and materialistic matter. Oxidation is the key reaction happening in the universe. Even though it is important in the cell life as well as non biosystems, yet times it might be unwanted reaction in some cases. One such case is release of more number of free radicals in a reaction in the biocell may lead to many unwanted cascade of reactions in the basic unit of life. These cascade of reactions may further give rise to reactions that cause cell damage in turn affecting the tissues of the organs and finally functioning of the organ. The present study was conducted to evaluate the antioxidant activity of the Polyherbal extract. The antioxidant activity was evaluated by following DPPH assay method. The standard used in this method was ascorbic acid.IC50 calculated from the data of the absorbance and percentage Inhibitory activity of Polyherbal extract was recorded as 20.06mcg/ml concentration where percentage Inhibitory activity of ascorbic acid was calculated as 26.74mcg/ml.

KEYWORDS: Antioxidant activity, DPPH, Polyherbal, Oxidation.

INTRODUCTION

Oxidation is mostly occurred reaction in nature. Excessive oxidation leads to release of large quantity of free radicals which are involved in many cascade reactions. The free radicals cause stress conditions in the cell leading to cell damage or cell death which in turn cause tissue and organ damage.[1] In order to avoid these reactions antioxidants were taken so that the scavenging of the free radicals occur there by protecting the cell functioning.

The antioxidant activity can be evaluated by many *invitro* as well *invivo* methods.[2] Mostly invitro studies were carried out to evaluate the antioxidant activity.[3] The *in-vitro* models available for determination of antioxidant activity are reducing power assay[4], hydroxyl radical scavenging method – using EDTA,$FeSO_4$,TCA etc., Nitric acid radical scavenging assay, superoxide radical scavenging assay,; 2,2- diphenyl-1- picryl-hydrazyl(DPPH) assay; 2,2' azino bis(3 – ethylbenzothiozoline-6-sulphonic acid)di ammonium salt($ABTS^{\cdot+}$) are the mostly used methods.[5]

The main principle behind all the assay methods is to decrease the free radicals released by the samples which is measured by spectrophotometer.

MATERIALS AND METHODS

Plant Material

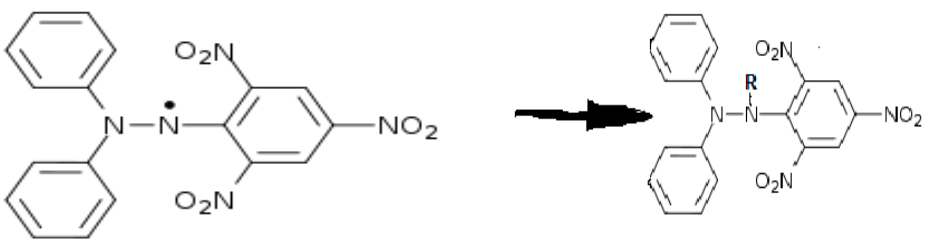
The authentificated plant material required for the activity was taken for the study which was certified by botanist Dr. Madhava Chetty. K, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.

Preparation of the extract

Polyherbal extract was prepared from the various parts of four different plant namely Acacia. ferruginea, Casearia elliptica, chloroxylon swietenia, Terminalia alata. All the plant parts were token in equal quantities to prepare the extract. Soxhlet extraction method was used to extract the constituents from the plants parts. Methanol is used as solvent. Extract was concentrated and stored in containers till the usage.

DPPH assay

2,2- diphenyl-1- picryl-hydrazyl is the compound which readily releases free radicals into the solution. It creates invitro condition of excessive oxidation on which test compound are tested for free radical scavenging activity.[6,7]



I II

The above reaction is the principle involved in antioxidant activity of DPPH. The compound I is the oxidized form of DPPH containing free radical on N which is taken up by scavenging activity of test compound added to DPPH solution. The solution of DPPH is violet in colour which turns into yellow after antioxidation.

Reagent required is DPPH, solvent used is methanol, standard used was Ascorbic acid and polyherbal extract.

DPPH solution preparation

4mg of DPPH which was procured fromsigma chemicals was dissolved into methanol(95%) of volume 100ml in dark room. The methanol is used as the solvent because the extract that is prepared from hot extraction was done by using methanol.

4mg of DPPH + 100ml of CH_3OH(methanol)

↓

0.004%w/v concentration of DPPH

The working standard concentration used for evaluation of activity is 0.004%w/v.

Test sample preparation: The methanolic dried extract of polyherbal extract of 4grams was taken and dissolved in 4ml of methanol. This makes the extract concentration of 1mg/ml.

4g of extract+4ml of methanol

↓

1mg/ml of Extract concentration

The above test solution was diluted to different concentration by serial dilutions. The different concentrations of the sample prepared were 10,25,50,100,200 mcg/ml. One ml of all the dilutions were taken in separate test tubes and 3ml of DPPH working stock previously prepared to added to each of the test tubes and kept aside for 30min and care show be taken that they were not exposed to light.

Ascorbic acid solution preparation: The standard for antioxidant activity taken in this study was ascorbic acid where the solution of the same is prepared by dissolving 2grams of ascorbic acid in 2.5ml of distilled water. The solution so formed is further diluted by serial dilutions to attain different concentrations of 10,25,50,100,200 µg/mL.

Blank preparation required is prepared adding 1ml of methanol in 3ml of DPPH solution.

Procedure

The above solutions were observed under UV-Visible spectrometer at the wave length of 517nm.One holder of the spectrometer received the blank and the other test compound i.e., extract or the standard. All the readings of absorbances were recorded in triplicate for each concentions of the study. The antioxidant activiyt was determined as the discolored DPPH due to test samples and calculated as % decolorized DPPH in the presence of test compound by formula as given below.

%Inhibition=(BA-ATS or STD/BA or A CTL) X100

BA=Blank absorbance

ABA= absorbance of the test sample or the standard

A CTL = Asorbance of the control

RESULTS

The DPPH which is used in evaluation of the activity, changes the occur of purple into yellow due to antioxidation carred out in the sample.

Percentage inhibition of the standard and Polyherbal extract.

conc.(µl/ml)	%Ascorbic acid	%polyherbal
10	43.848	42.61
25	49.589	65.49
50	53.916	75.65
100	74.794	84.48
200	90.486	97.39

The expressed are the average of triplicate values of each concentration.

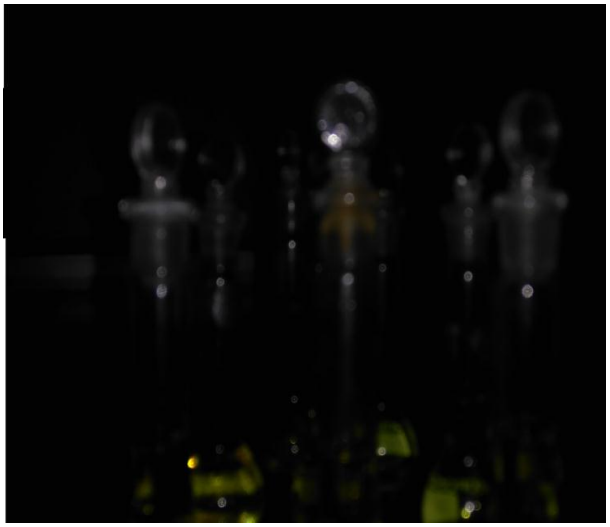
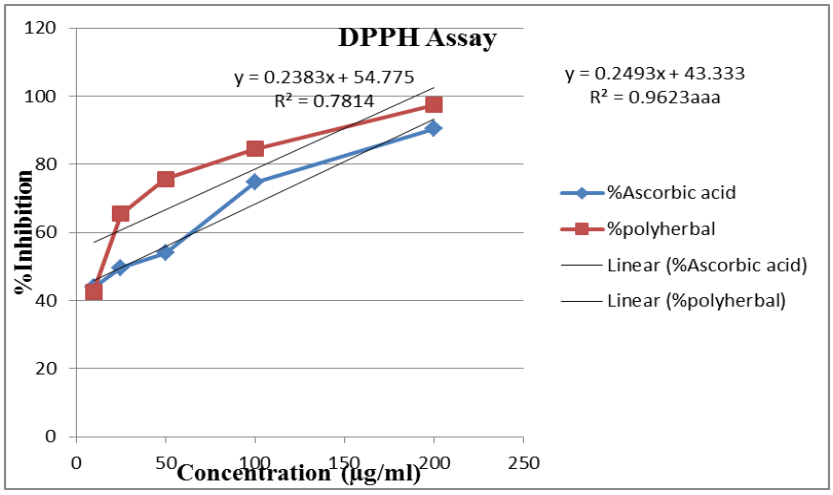


Fig. 1: Antioxidant activity of Polyherbal extract by DPPH assay.

The absorbances were recorded and percentage inhibition was calculated. The nonlinear graph was constructed by joining the points of test sample and also the standard. From the graph constructed, regression analysis was done,[8] IC50 was determined.



The IC50 calculated from the graphs were 20.06mcg/ml for Polyherbal extract and 26.3mcg/ml is the IC50 of the standard.

CONCLUSION

The antioxidant activity was determined by DPPH assay method. The absorbances of different concentrations of both the Polyherbal extract and standard were recorded and % Inhibition of free radicals were recorded. From the graph IC50 value was determined. 20.06mcg/ml for Polyherbal extract and 26.3mcg/ml is the IC50 of the standard ascorbic acid. This shows that at lowest concentration only polyherbal scavenges 50% of radicals when compared with the standard. This concludes that polyherbal extract have more potent antioxidant activity when compared with standard.

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