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Antioxidant screening methods in vitro for plant / herbal extracts.

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Screening of compounds for antioxidant property in vitro is most important for pharmacologist various methods are available many of them are modified the aim of the article is integrate different methods and to employ them for research purpose .In this article some important screening test are given for extract obtained from medicinal plants.

ABTS scavenging activity

The reaction was initiated by the addition of 1.0 ml of diluted ABTS to 10 µl of different concentrations of spagyric essence of the sample or 10 µl methanol as control . The absorbance will be read at 734 nm and the percentage inhibition will be calculated.

The inhibition will be calculated according to the equation

$I = A_1/A_0 \times 100$

where A_0 is the absorbance of control reaction, $A_1$is the absorbance of test compound.

Hydroxy radical activity

The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM $FeSO_4$, 0.7 ml of 6 mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate, and different dilution of the spagyric essence will be added. After incubation for 1 hour at 37°C, the absence of the hydroxylated salicylate complex will be measured at 562 nm. The percentage scavenging effect will be calculated as

Scavenging activity = $[1-(A_1-A_2)/A_0] \times 100\%$,

Where A_0 is absorbance of the control (without extract), A_1 is the absorbance in the presence of the extract, and A_2 is the absorbance without sodium salicylate.

Reducing power

The reaction mixture contained 2.5 ml various concentrations of spagyric essence of the sample, 2.5 ml of 1% potassium ferric cyanide and 2.5 ml of 0.2 M sodium phosphate buffer . The control contained all the reagents except the sample. The mixture will be incubated at 50°C for 20 minutes, and will be terminated by the addition of 2.5 ml of 10% (W/V) trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 minutes. 2.5 ml of the supernatant upper layer will be mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride, and absorbance will be measured at 700 nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicated increased reducing power of

the sample. Ascorbic acid will be used for comparison.

Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of NADH and phenazinemethosulphate (PMS) under aerobic condition .The 3.00 ml reaction mixture contained 50 µl of lM NBT, 150 µl of lM nicotinamide adenine dinucleotide (NADH) with or without sample and Trisbuffer (0.02 M, pH 8.0). The reaction will be started by adding 15µl of lM phenazinemethosulfate (PMS) to the mixture and the absorbance change will be recorded to 560 nm after 2 minutes. Percent inhibition will be calculated against a control without the extract.

Chelating activity

The reaction mixture contained 1.0 ml of various diluted spagyric essence , 0.1 ml of 2 mM $FeCl_2$ and 3.7 ml methanol. The control contained all the reaction reagents except the sample. The reaction will be initiated by the addition of 2.0 ml of 5 mM ferrozine. After 10 minutes at room temperature, the absorbance of the mixture will be determined at 562 nm against a blank. A lower absorbance of the reaction mixture indicated a higher iron chelating ability.

The capacity to chelate the ferrous ion will be calculated by

% chelation = $[1-(ABS_{sample}/ABS_{control}] \times 100$.

Ferric-reducing antioxidant power (FRAP) assay

The stock solution of 10 mM 2,4,6- tripyridyl-s- triazine (TPTZ) in 40mM HCL, 20 mM $FeCl_3.6H_2O$ and 0.3Macetate buffer (pH 3.6) was prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloridesolution and 25 ml acetate buffer. It will be freshly prepared and warmed to 37°C. FRAP reagent (900 µl) will be mixed with 90 µl water and 30 µl test ethanolic extract of the sample and standard antioxidant solution. The reaction mixture will be then incubated at 37°C for 30 minutes and the absorbance will be recorded at 595 nm. An intense blue color complex is formed when ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex was reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm will be recorded.

Scavenging of hydrogen peroxide

It can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. It can generate the hydroxyl radical in the

presence of metal ions and superoxide anion

$$O_2 + H_2O_2 \longrightarrow OH^- + OH^+ + O_2$$

A solution of hydrogen peroxide (20mM) will be prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the spagyric essence or

standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank
solution that contained extracts in PBS without hydrogen peroxide.

TBARS ASSAY

A modification of thiobarbituric acid reactive substances (TBARS) assay was used to determine the level of lipid peroxide formed using egg yolk homogenate as lipid-rich media . Egg homogenate (0.5 ml, 10% v/v) was added to 0.1 ml of extract (1mg/ml) and the volume made up to 1 ml with distilled water. Then, 0.05 ml of $FeSO_4$ was added and the mixture incubated for 30 minutes. Acetic acid (1.5 ml) and thiobarbituric acid (1.5 ml) in SDS was sequentially added. The resulting mixture was vortexed and heated at 95ºC for 60 minutes. After cooling, 5 ml of butanol was added and the mixture centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

Inhibition of lipid peroxidation (%) = (1 – E/C) x 100

Where C = absorbance of fully oxidized control and E = absorbance in the presence of extract.

Thanks to my teacher and department of pharmacology.