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Study on Antioxidant Activity of Different Extracts of *Andrographis paniculata* and *Asparagus racemosus*

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ABSTRACT

The aim of the study was to evaluate comparative antioxidant activity of different extract of *Andrographis paniculata* (AP) and *Asparagus racemosus* (AR). Simultaneously to observe the antioxidant effect of polyherbal formulation of both plant. In present study alcoholic and water extract of both plants was prepared and antioxidant activity was measured by nitric oxide scavenging activity and TBARS method (in vitro) and lipid peroxidation method (in vivo). Results indicate 1) Alcoholic extract of AR is more active than its aqueous extract. 2) Aqueous extract of AP is more active than its alcoholic extract. 3) Mixture of both alcoholic extract (polyherbal) show the high antioxidant activity compare to solo extract.

Keywords: Antioxidant, *Andrographis paniculata, Asparagus racemosus,* Polyherbal, TBARS, MDA, NO

1. INTRODUCTION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds then the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases [1]. India occupies the topmost position in the use of herbal drugs since ancient times utilizing nearly 600 plant species in different formulations [2]. The medicinal uses of *Andrographis paniculata* and *Asparagus racemosus* was reported from many years [3-7].

2. MATERIAL AND METHOD

The roots of AR and plant of AP were identified and authenticated from department of pharmacy B.U. Bhopal and collected locally from old city Bhopal. Extract prepared by cold maceration method. Animals: 30 healthy albino rats, weighted 180-200 gm were selected for the study. They were divided in to 10 groups, each contain 3 rats.

Assay for In Vitro Antioxidant activity

Nitric Oxide Scavenging Activity [8]

Sodium Nitroprusside (10 mM) in phosphate buffer saline was mixed with different concentrations of each extract (100, 200, 300, 400, 500µg/ml) dissolved in respective solvent and

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incubated at 25°C for 150 min. The same reaction mixture without extract but equivalent amount of solvent served as a control. After incubation period 1.5 ml of Griess Reagent (1% Sulphanilamide, 2% H3P04 & 0.1% Naphthyl ethelene diamine dihydrochloride {NEDA}) was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide subsequent coupling with NEDA was read at 564 nm. Curcumin was use as positive control.

% Inhibition = [1-Abs. of Sample/Abs. of Control] x 100

TBARS Method (Fenton reaction)

The antioxidant activity of extract was studied by their hydroxyl radical scavenging activity. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test copmpounds for hydroxyl radical generated by Fe^{2+}-EDTA- H_2O_2 (Fenton reaction). The hydroxyl radicals attack deoxyribose that eventually results in TBARS formation.

The reaction mixture consisted of 100 µl deoxyribose, 50 µl ferric chloride, 50 µl EDTA, l00 µl H_2O_2 in 550ul phosphate buffer saline. Different extract concentration, curcumin (positive control) or phosphate buffer saline was added to reaction mixture to make a final volume of l ml. The same reaction mixture without extract but equivalent amount of solvent served as a control. The reaction mixture was incubated for 1 h. at room temperature. Then the mixture was incubated for 20 min. in a boiling water bath with 0.5 ml. of 3 % of TCA and 0.5 ml. of 1% of TBA, cooled and centrifuged. The test tube with PBS was considered as blank. The absorbance of supernatant was measured at 532 nm in an UV spectrophotometer.

% Inhibition = [1-Abs. of Sample/Abs. of Control] x 100

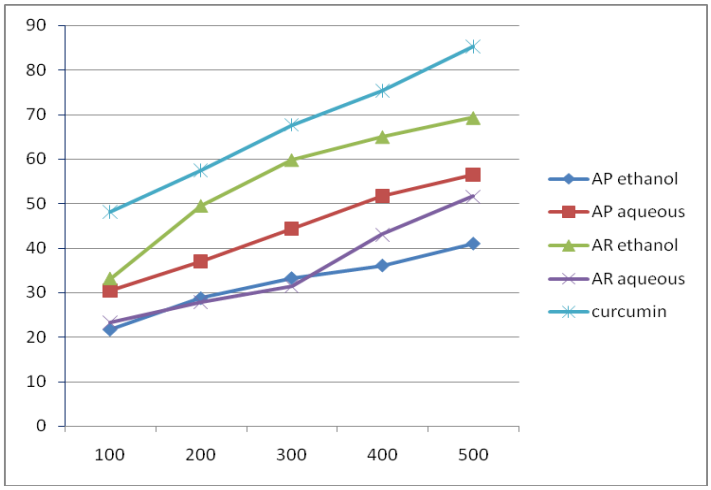
In vivo evaluation of antioxidant activity of alcoholic and polyherbal extract

Lipid peroxidation method

This procedure is followed by Kenjale et al [9] with minor modification. The lipid peroxidation was calculated on the basis of TBARS formation, which is the product of MDA and TBA reaction.

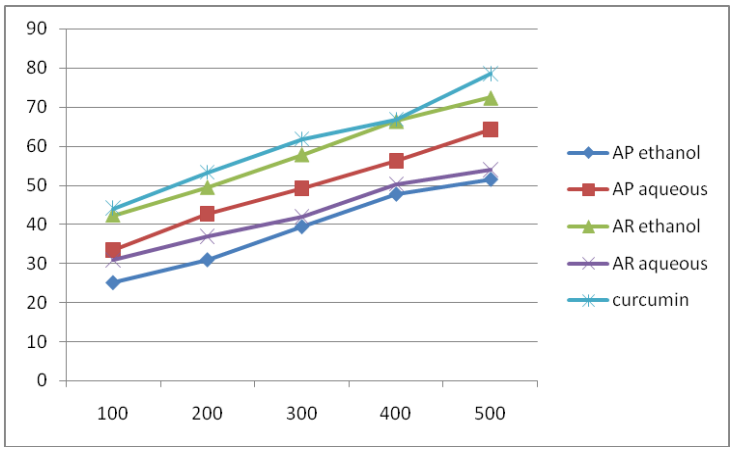
Experimental Result

In the present study all the extract shows increment in antioxidant activity with increased concentration. As illustrated by the figure and table 1, 2 and 3 increased concentration of each extract show the high % inhibition. Curcumin, which is used as a standard antioxidant, has the high antioxidant activity (% inhibition) compare to extracts.



Concentration (µg/ml) versus % inhibition

Figure 1: Nitric oxide scavenging activity



Concentration (µg/ml) versus % inhibition

Figure 2: Antioxidant activity by TBARS method

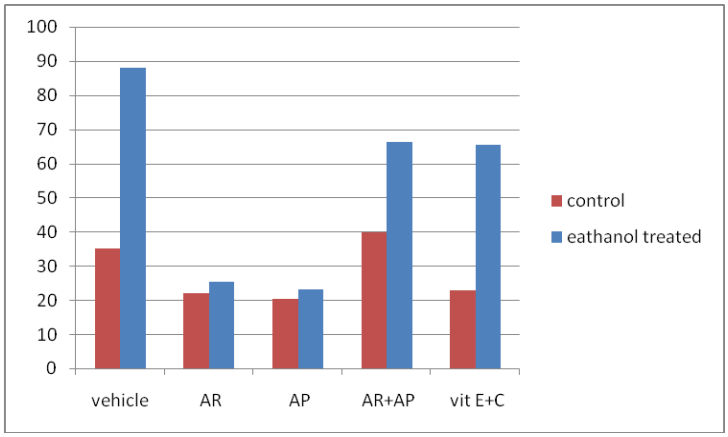


Figure 3: In vivo % inhibition versus different treatment

Table No 1: In vitro antioxidant activity by nitric oxide scavenging method

Conc (µg/ ml)	% inhibition by substances/ extracts				
	AP (ethanol)	AP (aqueous)	AR (ethanol)	AR (aqueous)	Curcumin
100	21.6±1.54	30.4±1.59	33.09±2 .34	23.23±12 .53	48.06±1 .09
200	28.67±0.6 1	37.01±1.21	49.45±1 .69	27.8±0.7 2	57.43±0 .57
300	33.17±0.9 6	44.27±1.06	59.76±1 .70	31.4±4.5 9	67.67±1 .31
400	36±1.59	51.67±1.40	64.96±0 .92	42.97±2. 58	75.37±2 .11
500	40.9±1.30	56.43±1.08	69.27±0 .64	51.58±0. 91	85.33±1 .58

Values are the mean ±SD of three observation (n=3), SD= standard deviation

Table No 2: In vitro antioxidant activity by nitric oxide scavenging method

Conc (µg/ ml)	% inhibition by substances/ extracts				
	AP (ethanol)	AP (aqueous)	AR (ethanol)	AR (aqueous)	Curcumin
100	25.17±1.9 9	33.43±0.86	42.3±1. 71	30.87±0. 76	44.03±1 .19
200	31±1	42.77±1.0	49.43±1 .21	37±0.91	53.23±0 .97
300	39.43±1.6 9	49.23±1.15	57.83±0 .40	42.03±0. 06	61.83±1 .39
400	47.73±0.7 6	56.16±0.67	66.36±0 .40	50.4±0.6 9	66.9±0. 1
500	51.5±0.43	64.2±1.21	72.4±1. 21	54.07±1. 01	78.63±0 .71

Values are the mean ±SD of three observation (n=3), SD= standard deviation

Table No 3: In vivo antioxidant activity of both alcoholic and polyherbal extract

Group	Treatment	% inhibition
Control	Vehicle	35 ± 1.25
	Asparagus racemosus	22.08 ± 2.6
	Andrographis paniculata	20.42 ± 0.78
	Polyherbal of AS+AP	39.75 ± 3.52
	Vit E+C	22.9 ± 1.91
Ethanol treated	Vehicle	87.9 ± 6.56
	Asparagus racemosus	25.41 ± 0.72
	Andrographis paniculata	23 ± 3.96
	Polyherbal of AS+AP	66.25 ± 1.24
	Vit E + C	65.41 ± 6.28

Values are the mean ±SD of three observation (n=3), SD= standard deviation

Table 3 represents the inhibition of MDA production by oxidation of polyunsaturated fatty acids with the help of extract and standard vitamins. The result indicates polyherbal formulation show the high antioxidant activity (approx vit E + C, which are standard), which is followed by AR and then AP.

3. RESULTS AND DISCUSSION

In vitro nitric oxide assay, sodium nitroprusside, gives nitric oxide which is a free radical. The chromophore formed during diazotization reaction of nitrite with sulphanilamide, subsequent coupling with NEDA (from griess reagent). Low intensity of color shows high activity of extract. In present study both plant and their both extract shows, nitric oxide scavenging activity.

In vitro TBARS assay free radical produced in Fenton reaction, degrade deoxyribose, and initiate a series of reaction that eventually result in the formation of thiobarbituric acid reactive substance, which forms a color product with TBA. When extract added in the reaction mixture, they scavenge the free radical and color is form of low intensity, show antioxidant activity of sample. Both extract of both plants decrease the intensity of the color, like standard curcumin. This fact illustrate that both plants have the good free radical scavenging activity.

The oxidative stress marker studies revealed that the chronic administration of ethanol, increased the level of lipid oxidation, decreased the activities of SOD and catalase, and reduced the content of GSH. Oxidative stress caused by ethanol possesses a significant correlation with lipid peroxidation and so increased level of malonlaldehyde (MDA).

The measurement of MDA, the decomposition product of oxidized polyunsaturated fatty acids, is commonly used as a method for the quantification of lipid peroxidation. In this method MDA form TBARS, which produced chromophore with TBA.

In this study observed that ethanol treated rats, has extensive generation of free radical, further administration of alcoholic extract of AP, AR and polyherbal (AR and AP), prevented the ethanol induced changes of oxidative stress and the effect was comparable to that of vit E and C.

On the basis of results, it was concluded that alcoholic extract of AR is more active than aqueous extract and simultaneously the aqueous extract of AP is more active than alcoholic extract. It was also observed that AR extract has more free radical scavenging activity compare to AP and the polyherbal extract prevent the ethanol induced changes, better than single extract. The observation suggests use of polyherbal formulation having synergistic effect.

Our results strongly suggest that both medicinal plants can be promising sources of potential antioxidants activity.

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