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Study on Antioxidant Activity of Different Extracts of *Withania somnifera* and *Asparagus racemosus*

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ABSTRACT

In the present study antioxidant activity of different extract of *Withania somnifera* (WS) and *Asparagus racemosus* (AR) was evaluated and compared. Simultaneously the antioxidant effect of polyherbal formulation of both plants also observed. The 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 both WS and AR are more active than its aqueous extract. 2) Mixture of both alcoholic extract (polyherbal) show the high antioxidant activity compare to solo extract.

Keywords: Antioxidant, *Withania somnifera, Asparagus racemosus*, Polyherbal, TBARS, MDA, NO

1. INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells.

India occupies the topmost position in the use of herbal drugs since ancient times utilizing nearly 600 plant species in different formulations [1]. The medicinal uses of *Withania somnifera* and *Asparagus racemosus* was reported from many years [2-6].

2. MATERIALS AND METHODS

2.1 Extraction

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

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2.2 Assay for *In Vitro* Antioxidant activity

2.2.1 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 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% H3PO4 & 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 used as positive control.

Table 1: In vitro antioxidant activity by nitric oxide scavenging method

Conc. (µg/ml)	% inhibition by substances/ extracts				
	WS (ethanol)	WS (aqueous)	AR (ethanol)	AR (aqueous)	Curcumin
100	35.53±1.50	26.6±0.72	33.09±2.34	23.23±12.53	48.06±1.09
200	37.9±0.85	34.27±1.00	49.45±1.69	27.8±0.72	57.43±0.57
300	41.87±2.42	38.4±0.79	59.76±1.70	31.4±4.59	67.67±1.31
400	52.17±2.25	43.97±3.09	64.96±0.92	42.97±2.58	75.37±2.11
500	63.73±0.75	45.67±1.47	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

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

2.2.2 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 Fe2+-EDTA- H2O2 (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 H2O2 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

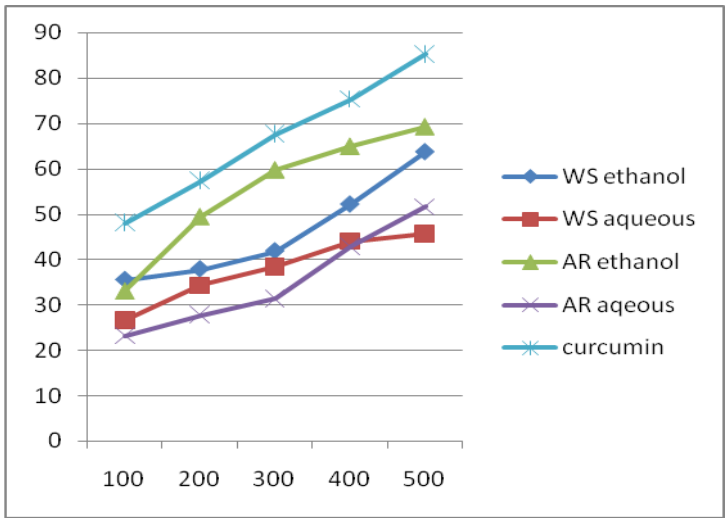

Fig 1: Nitric oxide scavenging activity

Table 2: In vitro antioxidant activity by TBARS method

Conc (µg/ml)	% inhibition by substances/ extracts				
	WS (ethanol)	WS (aqueous)	AR (ethanol)	AR (aqueous)	Curcumin
100	42.2±1.35	33.56±1.42	42.3±1.71	30.87±0.76	44.03±1.19
200	48.4±2.42	37.06±0.46	49.43±1.21	37±0.91	53.23±0.97
300	55.13±1.30	40.53±0.61	57.83±0.40	42.03±0.06	61.83±1.39
400	62.35±1.79	43.2±0.44	66.36±0.40	50.4±0.69	66.9±0.1
500	69.8±1.05	48.53±1.41	72.4±1.21	54.07±1.01	78.63±0.71

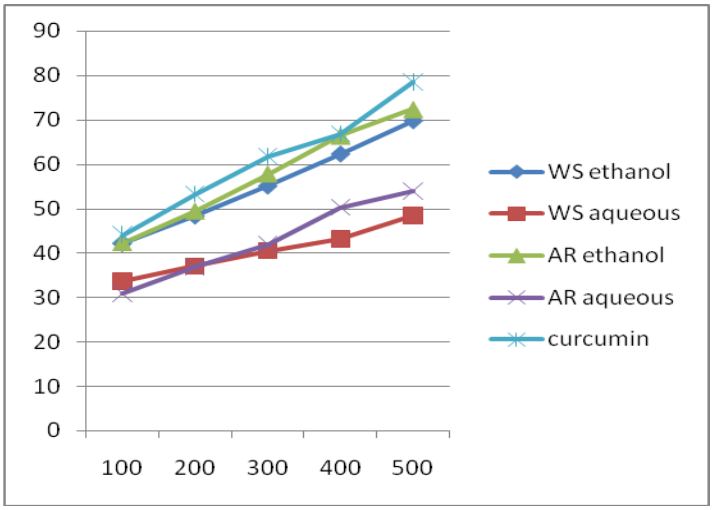

Fig 2: antioxidant activity by TBARS method

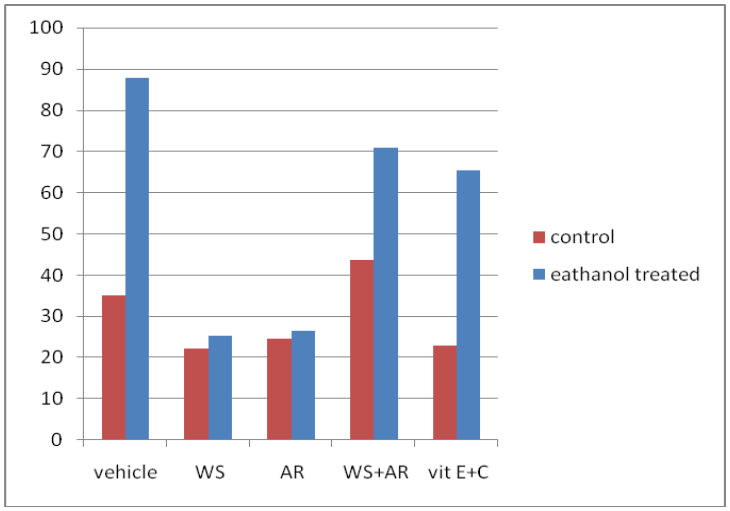

Fig 3: In vivo % inhibition versus different treatment

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 the polyherbal formulation of both extracts (ethanolic) shows the high antioxidant activity (approx vit E + C, which are standard).

Table 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
	Withania somnifera	24.5± 0.56
	Polyherbal of AS+AP	43.73 ± 0.25
	Vit E+C	22.9 ± 1.91
Ethanol treated	Vehicle	87.9 ± 6.56
	Asparagus racemosus	25.41 ± 0.72
	Withania somnifera	26.57 ± 1
	Polyherbal of AS+WS	70.83 ± 0.25
	Vit E + C	65.41 ± 6.28

2) 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.

3. RESULTS AND DISCUSSION

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

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 WS, AR and polyherbal (WS and AR), prevented the ethanol induced changes of oxidative stress and the effect was comparable to that of vit E and C.

4. CONCLUSION

On the basis of results, it was concluded that alcoholic extracts of WS and AR is more active than aqueous extracts. The study demonstrates high in vitro antioxidant activity of AR compared to WS. But it was also observed that WS extract has more free radical scavenging activity compare to AR in vivo, which suggests WS works as good free radical scavenger in vivo. 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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