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Antioxidant potential of ethanolic extracts of Arisaemia jacquemontii

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ABSTRACT

Oxygen free radicals include damage due to peroxidation to biomembranes and also to DNA, which lead to tissue damage, thus cause occurrence of a number of diseases. Antioxidants neutralise the effect of free radicals through different ways and may prevent the body from various diseases. Antioxidants may be synthetic or natural. Synthetic antioxidants such as Butylated hydroxytoulene (BHT) and Butylated hydroxyanisole (BHA) have recently being reported to be dangerous for human health. Thus, the search for effective, non toxic natural compounds with antioxidative activity has been intensified in recent years. Objective of the study is to explore antioxidant potential of Arisaemia jacqumontii.

Key words: In vitro antioxidant, Lipid peroxidation.

I. INTRODUCTION

Present study has the aim to determine the phytochemical screening, total phenolic content, DPPH Assay, Hydroxyl Radical Scavenging activity, Reducing power and Lipid peroxidation of ethanolic extract of *Arisaemia Jacquemontii*.

Harmful intermediates called reactive oxygen species (ROS) are produced during biological combustion involved in the process of respiration which include ROS like superoxide anions, hydroxyl radicals, hydrogen peroxide, alkoxyl radicals that cause damage to the biological macromolecules (DNA, proteins, lipids, carbohydrates etc.) resulting in various degenerative diseases like cancer, atherosclerosis, cardiovascular diseases[1]. The balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system [2]. Dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage [3, 4]. Because of potential health risk and toxicity associated with synthetic antioxidants a widespread agreement needed to replace the synthetic antioxidants with natural antioxidants [5]. Therefore, the search for antioxidants from natural sources has received much attention, and efforts have been made to identify new natural resources for active antioxidant compounds. Several assays have been frequently used to estimate antioxidant capacities in plant extracts including DPPH, reducing power, superoxide radical, hydrogen peroxide radical, hydroxyl radical and lipid peroxidation assays[6]. These techniques have shown different results among plants tested and across laboratories [7].

II. MATERIAL AND METHODS

II.1, Plant material collection

The whole plant *Ariseamia Jacquemontii* was collected in the month of August from Doodhpathri, District Budgam, Jammu and Kashmir and identified by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir, and identified by Akhter Hussain Malik. A reference specimen has been retained in the herbarium under reference number KASH.bot/KU/AJ-703-IA.

II.2, Extraction

The plant material was dried in the shade at 30 ± 20˚C. The dried material was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted in ethanol solvent using a Soxhlet extractor (60-80˚C). The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for future use.

III. PHYTOCHEMICAL SCREENING OF THE CRUDE EXTRACTS

Phytochemical screening was performed by using various standard procedures.

IV. ASSESSMENT OF ANTIOXIDANT ACTIVITY

IV.1, DPPH Radical Scavenging Activity: The DPPH assay was performed by using the method of Braca [8]. Various concentrations of plant extracts (100-600µg/ml were added to 1ml of the 0.004% methanol solution of DPPH, and the mixture was vortexed vigorously. The tubes were then incubated at room temperature for 30 minutes in dark, and the absorbance was taken at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

IV.2, Total phenolics: The total phenolics in different extracts were determined by using Folin-Ciocalteau reagent according to the protocol of Chandler [9]. Quantitation was based on the standard curve of Gallic acid (10mg %), which was dissolved in methanol/water (60:40, v/v, 0.3% HCl). The concentration of polyphenols was expressed in terms of mg/100ml of sample.

IV.3, Reducing power: The reducing power of plant extracts were evaluated according to Oyaizu [10]. Different concentrations of the plant extracts were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium hexacyanoferrate. The mixture was incubated at 50^0C for 20 minutes, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700nm.

IV.5, Assessment of Hydroxyl radical scavenging property: Hydroxyl radical, generated from the Fe^{3+}- Ascorbate- H_2O_2 (Fenton reaction), were evaluated by degradation of deoxyribose that produced thiobarbituric acid reactive species (TBARS) [11]. The reaction mixture containing 25mM deoxyribose, 10mM Ferric chloride, 100mM ascorbic acid, 2.8 mM H_2O_2 in 10mM KH_2PO4 (pH 7.4) and various

concentrations of plant extracts. The reaction mixture was incubated at 37°C for 1h. Then one ml of 1% thiobarbituric acid and 1 ml of 3% trichloroacetic acid was added and heated at 100°C for 20 min. The TBARS were measured spectrophotometrically at 532 nm.

IV.6, Preparation of liver microsomes

Liver from the freshly killed rats were perfused and kept in an ice cold normal saline 0.9% NaCl and extraneous material was removed. All operations were performed at 4°C. Tissue was blotted between the folds of a filter paper and weighed. 20% (w/v) homogenate was prepared in 0.25M sucrose. The homogenate was filtered through a muslin cloth and centrifuged at 12000 g for 20 min at 4°C to separate nuclear debris. The supernatant so obtained was diluted 1:5 with 0.125 M sucrose containing 8 mM CaCl2 and kept on ice for 50 min with constant stirring. The pellet obtained after centrifugation at 12000 g for 10 min was washed with the washing solution containing 0.15 M KCl, 1 mM EDTA and 0.01 M NaH2PO4 and was again centrifuged at 12000 g for 10 min to get the microsomal pellet.

The microsomal pellet was then resuspended in a minimum volume of 0.25 M sucrose and stored at -80°C for experimental use.

IV.7, Lipid peroxidation assay (Liver Microsomes)

The assay of lipid peroxidation was done using the method of Chang *et al.*[12] Liver microsomes were incubated for 5 min in presence and absence of plant extract (50 µ-1000 µg) prior to addition of 100 µM FeSO4 and 50 µM H2O2 and then incubated for 20 min (37°C) in 0.15 M NaCl (pH 7). Control incubation received vehicle only and the induced incubation contained vehicle plus liver microsomes but had no addition of plant extract. The reaction was terminated by the addition of TCA-TBA reagent (5% w/v) and the lipid peroxidation content of the samples was determined as malondialdehyde (MDA) formed per mg of protein at 532 nm absorbance. Percentage inhibition was calculated using the formula;

% inhibition=1–(Induced-Treated/Induced-Control) × 100

V. RESULTS

V.1, Phytochemical screening of Arisaemia jacquemontii extracts

The results of the phytochemical analysis of the of Arisaemia jacquemontii in ethanolic solvent has shown a remarkable variation in phytochemical compounds. The detailed investigations of phytochemicals in ethanolic solvents are shown in Table 1. The study revealed that the ethanolic extract of *Arisaemia jacquemontii* are showing maximum presence of Flavanoids, Saponins, Phenols, Volatile oil and Cardiac glucose. However Alkaloids, Terpenoids, Phylobatanins, Cardenolides, Steroids and Anthraquinones lacked in ethanolic extract of *Arisaemia jacquemontii*.

Table 1.

Phytochemical Analysis of Ethanolic Extract of Arisaemia jacquemontii

Parameters	Ethanolic Extract
Alkaloids	–
Flavanoids	+
Saponins	+
Phenols	+
Terpenoids	–
Phylobatanins	–
Volatile oil	+
Cardenolides	–
Steroids	–
Anthraquinones	–
Cardiac glucose	+

V.2, DPPH Radical Scavenging Activity:

The reactivity of ethanolic extract of *Arisaemia jacquemontii* DPPH, a stable free radical. As DPPH picks up one electron in the presence of a free radical scavenger, the absorption decreases and the resulting discoloration is stechiometrically related to the number of electrons gained [13]. The DPPH Radical Scavenging [%] Activity is shown in the Fig.1, *Arisaemia jacquemontii* extract exerted an inhibition of 75.36% and that of Ascorbic Acid (Standard) was 83.4%. Half inhibitory value (IC50) of Arisaemia jacquemontii was 350 µg/ml.

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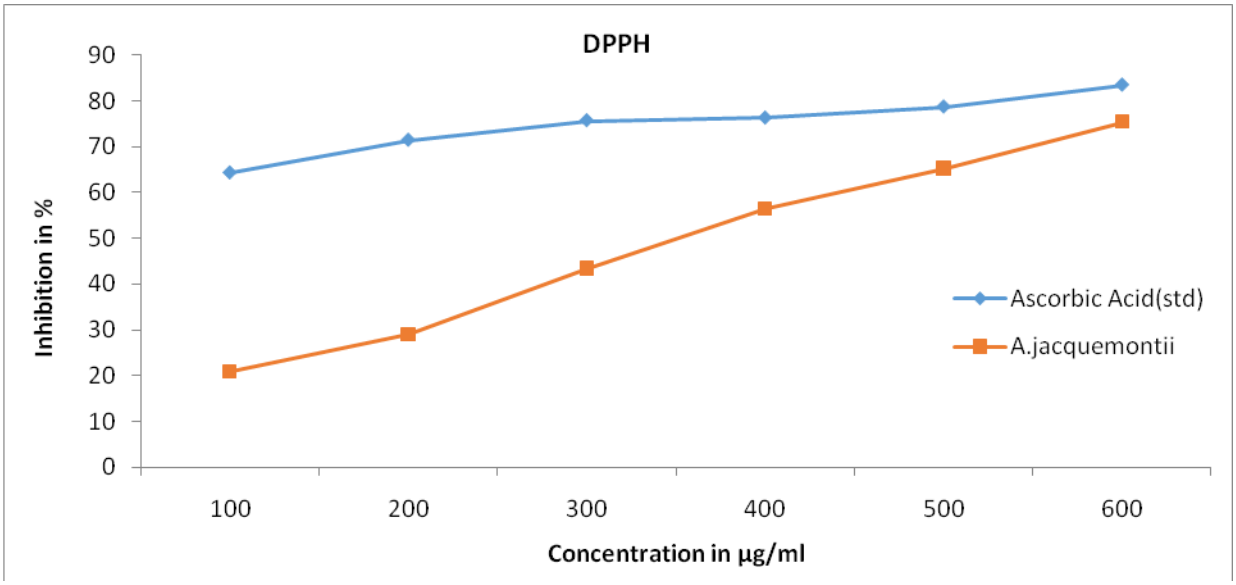

Figure 1

V.3, Total Phenolics:

Phenols are very important plant constituents and because of the presence of hydroxyl groups they have a great scavenging ability. Phenolic compounds are, therefore, known to be powerful chain breaking antioxidants. Ethanolic extracts of Arisaemia jacquemontii showed a significant total Phenolic concentration.

V.4, Reducing Power Assay:

The antioxidant can donate an electron to free radicals, which leads to the neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of $Fe_3(CN^-)_6 - Fe_2+(CN^-)_6$ [14]. The product was visualized by forming the intense Prussian blue colour complex and then measured at 700nm. Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and un reactive species. Reducing power capability of ethanolic extract of Arisaemia jacquemontii was excellent and increased with dose dependent manner. As shown in (fig. 2), a higher absorbance value indicates stronger reducing power of the sample. The reducing power activity of ethanolic extract at 100-600 ug/ml was increased from 0.04 to 0.14 respectively.

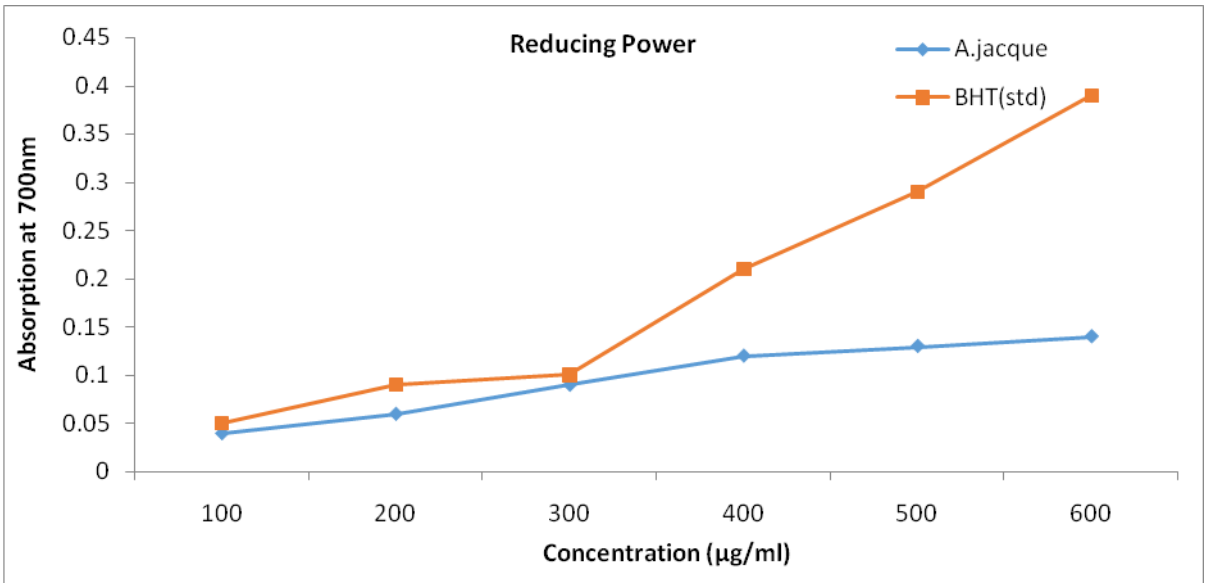

Figure 2

V.6, Assesment of Hydroxyl Radical Scavenging Activity

A significant decrease in the concentration of hydroxyl radical was observed due to Arisaemia jacquemontii extract. Ethanolic extract exhibited significant activity in a concentration dependent manner with an inhibition of 39.13% (Fig.3) and half inhibitory concentration (IC50) was 128µg/ml . BHT used as a standard inhibited the hydroxyl radical scavenging up to 47.8% with the same concentration.

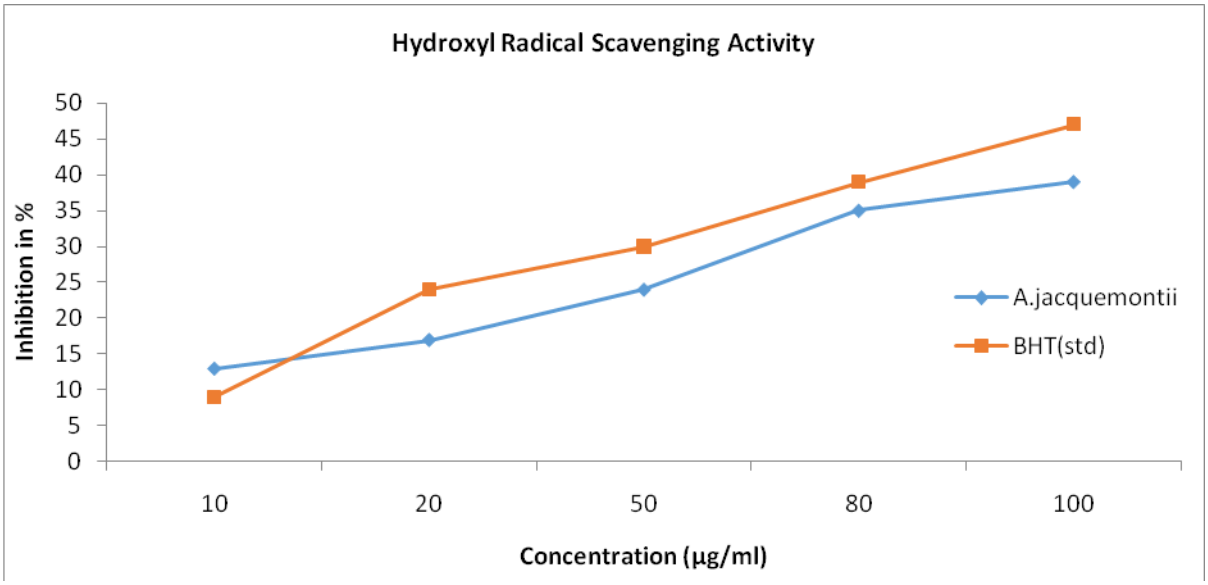

Figure 3

V.7, Microsomal lipid peroxidation of ethanolic extract of Arisaemia jacquemontii.

Antioxidant capacity of *Arisaemia jaquemontii* in the biological lipid peroxidation system was examined using rat liver microsomes. Addition of FeSO4 and H2O2 to the microsomal suspension increased TBARS time-dependently up to 20 min. When the microsomes had been pre-treated with each tested extracts, the production of TBARS was inhibited markedly in a dose dependent manner. At the high concentration of the plant extracts (700 µg/ml), we observed about 85% inhibition in TBARS formation with ethanolic extract. The half inhibitory concentration (IC50) of ethanolic extract in this system was 500µg/ml. BHT a know antioxidant used in the study inhibits the TBARS formation up to 96.85% at the concentration of 700 µg/ml.

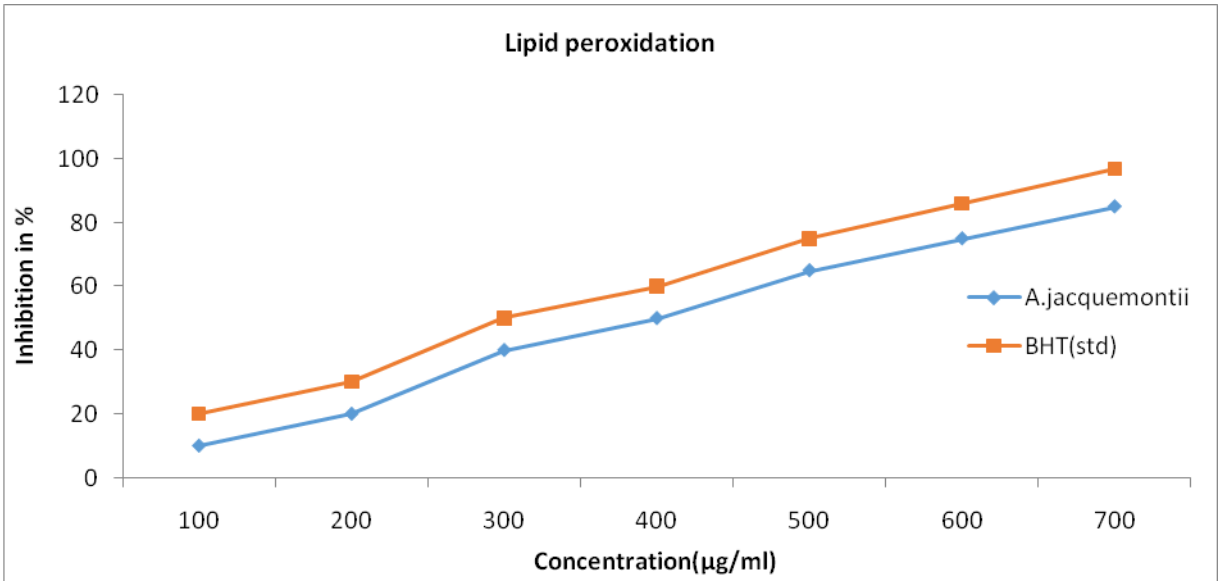

Figure 4

VI. CONCLUSION

Overall, it could be concluded that Arisaemia Jaquemontii possesses a potent antioxidant activity. The ethanolic extract of *Arisaemia jacquemontii* was found to be an effective scavenger of DPPH and showed high Radical scavenging activity. It possessed a good reducing power and exhibited a significant total phenolic content. High inhibitory concentration of 85% was seen in biological Lipid peroxidation system. Additional studies are needed to characterize the active compounds and to clarify the *in vivo* potential of this plant.

VII. ABBREVIATION USED

LPO: Lipid peroxidation; **DPPH:** 1, 1-Diphenyl-2-picryl-hydrazyl **ROS:** Reactive oxygen species; **GAE:** Gallic acid; **BHT:** Butylated hydroxytoluene; **TPC:** Total phenolic content; **MDA:** Malondialdehyde; **H_2O_2:** Hydrogen peroxide.

REFERENCES

[1.] Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009; 417(1):1-13.
[2.] Halliwell B. Oxidative stress and neuro degeneration: where are we now?.*J Neurochem*. 2006;
[3.] 97(6):1634-58
[4.] Sca lbert A, Johnson IT, Saltmarsh M. Polyphenols: Antioxidants and beyond. *American J Clin*
[5.] *Nut.*2005; 81(1):215-7.
[6.] Blokhina O, Virolainen E, Fagersted KV. Antioxidants, oxidative damage and oxygen deprivation
[7.] stress: a review. *Annals Bot.* 2003;91(2):179-94.
[8.] Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. *British J Nut.* 2001;85(s2): 67-74.
[9.] Wong SP, Leong LP, Koh JHW. Antioxidant activities of aqueous extracts of selected plants. *Food*
[10.] *Chem*. 2006;99(4):775-83.
[11.] Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH. Comparison of ABTS, DPPH,
[12.] FRAP and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Comp*
[13.] *Anal*. 2006;19(6):669-75.
[14.] Braca, A., De Tommasi Nunziatina, Di Bari Lorenzo, Pizza Cosimo, Politi Mateo and Morelli
[15.] Ivano (2001). Antioxidant principles from Bauhinia terapotensis, *Journal of Natural products* 64,
[16.] 892-895.
[17.] Chandler, S. F and Dodds, J. H (1993). The effect of phosphate, nitrogen and sucrose on the
[18.] production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell*
[19.] *Report*. 2, 1005-110.
[20.] Oyaizu, M (1986). Studies on products of browning reaction prepared from glucosamine.
[21.] *Japanese Journal of Nutrition* 44, 307-315.
[22.] Halliwell, B., Gutteridge, J. M and Aruoma, O. I (1987). The deoxyribose method: a simple "test
[23.] tube" assay for determination of rate constants for reactions of hydroxyl radicals, *Analytical*
[24.] *Biochemistry* 165, 215-219.
[25.] Chang ST, WU JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activities of extracts from
[26.] *Acacia confuse* bark and heartwood. *J Agric Food Chem*. 2001; 49(7):3420-4.
[27.] Silva CG. Herdeiro RS, Mathias CJ, Panek AD, Silveira CS, Rodrigues VP*l*. evaluation of
[28.] antioxidant activity of Brazillian plants. *Pharmacol*. Res 2005; 52, 229-233.
[29.] Yen GC, Chen HY. Antioxidant activity of various tea extracts in relation to their antimutagenicity.
[30.] *Journal of Agriculture and Food chemistry* 1995; 43, 27-32.
[31.] Pramod Kumar, Deval Rao, Lakshmayya, Ramachandra Setty. Antioxidant and hepatoprotective
[32.] activity of tubers of *Momordica tuberosa* cogn, against CCl4 induced liver injury in rats. *Indian*
[33.] *Journal of Experimental* Biology 2008; 46, 510-513.
[34.] Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI. Screening of antioxidant and antimicrobial
[35.] activities of anise (Pimpinella anisum L.) seed extracts. *Food chemistry* 2003; 83, 371-382