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Establishing the Antioxidant Activity of the Ethanol Extract of Adathoda Vasica

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Abstract: ***Antioxidants act aschiefprotectants against radical mediated toxicity to secure the damages induced by free radicals. Antioxidant established medications are often used to prevent and treat few complex diseases like diabetes, cancer and asthma. Thus an experiment was carried out to determine the percentage of antioxidant activity and lipid peroxidation of Adathodavasica against respiratory isolates. Hence the, ethanol extract of A.vasica was screened for invitro antioxidant activity by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity. The results of the extract were compared with butylatedhydroxytoluene (BHT) used as a standard. A concentration dependent response was found in the DPPH radical scavenging capacity and the activity was increased with the increase of sample concentration. A. vasica extract showed maximum of 58% inhibition of DPPH radicals at concentration of 10mg/ml, while BHT maintained as control showed about 93% inhibition at concentration of 1mg/ml respectively, Hence we conclude that the ethanolic extract of A. vasica possess antioxidant property to inhibit the oxidation of other molecules which can act as a defense mechanism and prevent the target bacteria from damage.***

Key Words: Antioxidant, Adathodavasica, DPPH, free radical, BHT

I. INTRODUCTION

Free radicals produced through several metabolic activities cause oxidative damage of tissues and it ultimately leads to degenerative diseases. Reactive oxygen species (ROS), also said to be active oxygen species (AOS), occur as different forms of activated oxygen, which consist of free radicals such as super oxide ions, hydroxyl radicals and it covers non free radical species like hydrogen peroxide (Hancock JT,2001). Commercially available antioxidant compounds, such as butylatedhydroxytoluene (BHT) are frequently used, but it has been reported to cause few side effects (Shahidi F,1992). To overcome this problem, several plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties. Natural medicines are very easy available to a human being and also it was economical. Synthetic medicines are replaced by natural medicines of plant origin. Among several plants used, A.vasica is one plant used in ayuervedic medicine, but has not been experimentally validated. Adathodavasica belongs to the family Acanthaceae. It is an erect, terrestrial, perennial shrub. The leaves are dark green above and pale yellow below. The flowers are typical, white, arranged in a pedunculated spike. Adathodavasica (Acanthaceae) commonly known as vasaka distributed throughout India up to an attitude of 1300m (Gupta O.P, 1977). The leaves, flowers, fruit, and roots are extensively used for treating cold cough, whooping cough, chronic bronchitis and asthma as sedative, expectorant and antispasmodic by the tribals. The plant is recommended for a variety of ailments such as bronchitis, asthma, fever, jaundice etc. The leaves & roots are efficacious in coughs, arthritis, diarrhoea and dysentery have the best chemostatic quality. Leaves are anti-inflammatory, and effective in skin disorders and cardiotonic. Essential oils of the leaves of A. vasica are also known to contain ketone, terpene, and phenolic ether which have antioxidant activity (Ignacimuthu,2010). The present study was undertaken to determine the percentage of antioxidant activity in ethanol extract of A.vasica using DPPH against target respiratory isolate K. pneumoniae.

II. MATERIALS AND METHODS

A. Preparation of plant material and extract

Leaves of Adathodavasica were collected from local market at Tiruchirappalli District, Tamilnadu, India and the plant was authenticated by the Botany department of St. Joseph's College and the voucher specimen(A001) was obtained. The extract was prepared as already mentioned
(Sharly N, 2016), and further dilutions (10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, 0.08, 0.04 & 0.02 mg/ml) were prepared using ethanol. A different concentration of butylatedhydroxytoluene (BHT) was used as a positive control.

B. PPH radical scavenging activity (Sanchez-Moreno et al.,1998)

Different concentrations of the extract (100µl) were added to 0.9 ml of methanolic solution of DPPH (2.5mg/100ml) and the reactants were incubated at room temperature for 30 min in dark. Different concentrations of butylatedhydroxytoluene (BHT) were used as a standard and the solvent (ethanol) was used instead of extract in control. After 30 min, the absorbance was measured at 515nm using a spectrophotometer and the radical scavenging activity of the extract was calculated and expressed on percentage basis.

C. Lipid peroxidation inhibition (Botsoglu et al.,1994)

Goat brain was collected from freshly sacrificed animal in local slaughter house and kept in phosphate buffer saline (PBS) under cold condition until analysis. Tissue was cut by using sterile blade and weighed 500 mg and then homogenized with 5 ml PBS buffer (pH 7.0). The brain homogenate (0.3ml) was pre-incubated with 0.3 ml of plant extract (1mg/ml) and then 0.1ml of 3% H_2O_2 was added and incubated at 37^{o}C for 10 min. BHT (1mg/ml) was used as positive control while 0.3 ml of PBS was used instead of extract in control. The reaction was stopped by adding 1.5 ml of TBA reagent (0.375 g of TBA dissolved in 10% TCA). The contents were then heated at 80^{o}C for 20 min, cooled down to room temperature and centrifuged at 3000 rpm for 10 minutes. The absorbance of supernatant was measured at 530 nm and based on the OD value the MDA level and lipid peroxidation inhibition capacity of the sample was calculated.

III. RESULTS

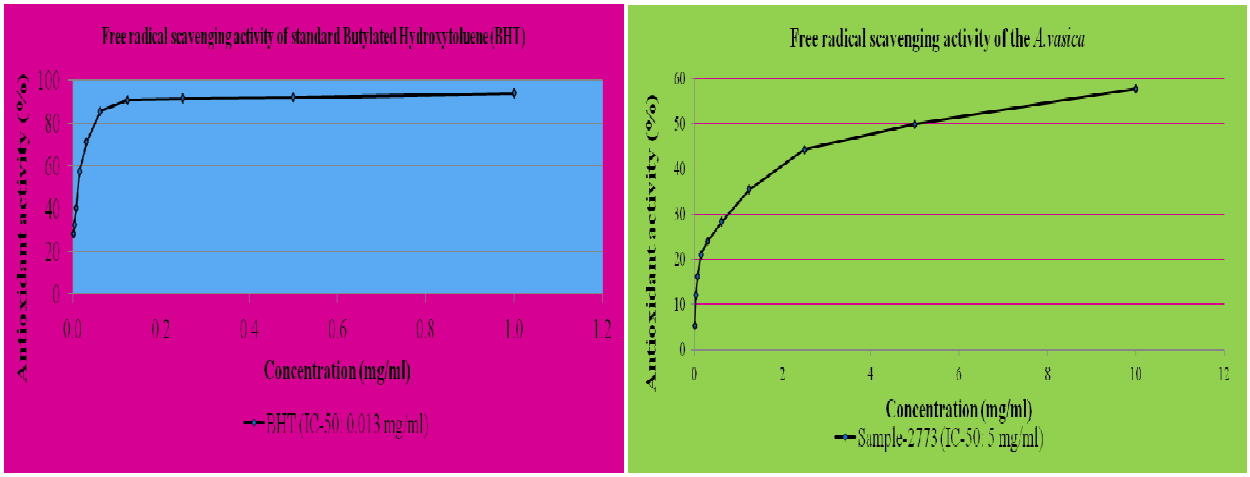
A. Antioxidant assay –DPPH method

Oxidative stress is the main cause for chronic degenerative diseases. During oxidation, free radicals are formed which damage the cells (Bus JS,1979). Antioxidativedefence mechanism is necessary to diminish the action of free radicals. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow coloured α,αdiphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant. (Table1) shows the percentage of DPPH radical scavenging capacity of ethanol extract of A.vasica in comparison with standard antioxidant like butylatedhydroxy toluene (BHT). A concentration dependent relationship was found in the DPPH radical scavenging capacity and the activity was increased with the increase of sample concentration. A.vasica extract showed maximum of 58% inhibition of DPPH radicals of IC=50 value at concentration of 10mg/ml, while BHT was maintained as control and it showed about 93% inhibition at concentration of 1mg/ml respectively.

Table.1: Antioxidant activity of A.vasica by DPPH method

S. No.	Standard (BHT)		*A.vasica* extract	
	Concentration (mg/ml)	Antioxidant activity (%)	Concentration (mg/ml)	Antioxidant activity (%)
1	1	93.98 ± 1.70	10	57.83 ± 3.41
2	0.5	92.17 ± 4.26	5	50.00 ± 0.43
3	0.25	91.57 ± 5.11	2.5	44.38 ± 6.68
4	0.1250	90.96 ± 4.26	1.25	35.54 ± 2.13
5	0.0625	85.54 ± 0.01	0.625	28.31 ± 0.43
6	0.0313	71.08 ± 1.15	0.3125	24.10 ± 0.85
7	0.0156	57.23 ± 3.97	0.1563	21.08 ± 1.28
8	0.0078	40.36 ± 4.26	0.0781	16.27 ± 0.43
9	0.0039	32.53 ± 1.51	0.0391	12.05 ± 0.85
10	0.0020	28.31 ± 4.26	0.0195	05.42 ± 0.43
	IC-50 Value	0.013 mg/ml	--	5 mg/ml

Fig.1: Antioxidant activity of *A.vasica* by DPPH method



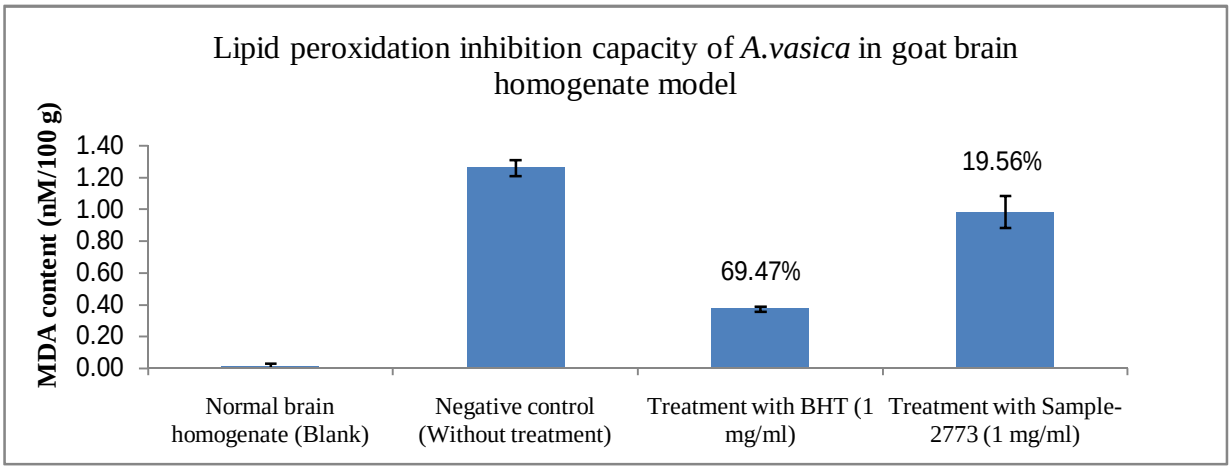
B. Lipid peroxidation inhibition

In present study this assay used to determine the potential of ethanol extract of A.vasicainhibit lipid peroxidation in goat brain homogenate, induced by PBS (Fig 2) at the concentration of 1mg/ml. In assessment the 1mg/ml concentration of the extract has the potential to inhibit the MDA content of lipid peroxides up to 20% respectively and it was compared with the control BHT. In the present investigation it is interestingly observed that ethanol extract of A. vasica showed 19.56% inhibition of LPO. As a result the presence of alkaloids in the extract plays a vital role as a potent inhibitors of malondialdehyde (MDA) at the range of 0.37 ± 0.02 for BHT and 0.99 ± 0.10 for extract respectively Thus the MDA and LPO reacts with TBA and TCA to inhibit the enzyme lipid peroxidases significantly at 1mg/ml concentration of A.vasica extract.

Table.2: Inhibitory effect of Lipid peroxidation of A.vasica

S. No.	Name of the sample	MDA content (nM / 100 g tissue)	Lipid peroxidation inhibition (%)
1	Normal brain homogenate (Blank)	0.01 ± 0.00	--
2	Negative control (Without treatment)	1.26 ± 0.05	--
3	Treatment with BHT (1 mg/ml)	0.37 ± 0.02	69.47%
4	Treatment with extract *A.vasica* (1 mg/ml)	0.99 ± 0.10	19.56%

Fig.2: Inhibitory effect of Lipid peroxidation of A.vasica



IV. DISCUSSION

The antioxidant activity of methanol extract of A. vasica leaves using BHT as standard has been reported by (Ignacimuthu & Shanmugam, 2010). The radical scavenging activity was the maximum at 1000μg/ml (66.15%). Other reports reveals that the extract of A. vasica showed presence of high levels of polyphenolic compounds (phenolic compounds and flavonoids), which could be the possible reason behind the antioxidant activity of the plant (RaoBhaskar,2011). The enzymatic and non-enzymatic antioxidants in A.vasica were found to be significant, similarly the antioxidant and radical scavenging activities of A.vasica were found to bemore promising (Padmaja M, 2011). The ethanolic extract of A. vasica has demonstrated antioxidant property to inhibit the oxidation of other molecules which can be act as a defense mechanism and prevent the target cells from damage.LPO is one of the main manifestations of oxidative damage initiated by ROS and it has been linked to the altered membrane structure and enzyme inactivation. The increase in LPO reported here, may be the result of increased production of free radicals (Barry H,1989).It is well known that decrease in the activities of antiperoxidative enzymes and increase in the content of lipid peroxidation results in cell damage (Livingstone DR,1990). The presence of alkaloids in the extract (Singh RP, 2000) plays a vital role as potent inhibitors of malondialdehyde (MDA). Reports revealed the inhibition of FeSO4 induced lipid peroxidation of *A. vasica* was found to be (6.2±4.809%) at 1mg/ml of the extract (Hemalatha K, 2011).

V. CONCLUSION

In assessment the 1mg/ml concentration of the extract has the potential to inhibit the MDA content of lipid peroxides up to 20% respectively and it was compared with the control BHT. In the present investigation it is interestingly observed that ethanol extract of A.vasica showed 19.56% inhibition of LPO. It is hence presumed that the phytocompounds in the extract play a vital role as a potent inhibitors of malondialdehyde (MDA) at the range of 0.37 ± 0.02 for BHT and 0.99 ± 0.10 for extract respectively Thus the MDA and LPO reacts with TBA and TCA to inhibit the enzyme lipid peroxidases significantly at 1mg/ml concentration of A.vasica extract. Thus A. vasicais known to possess remarkable antioxidant activity.

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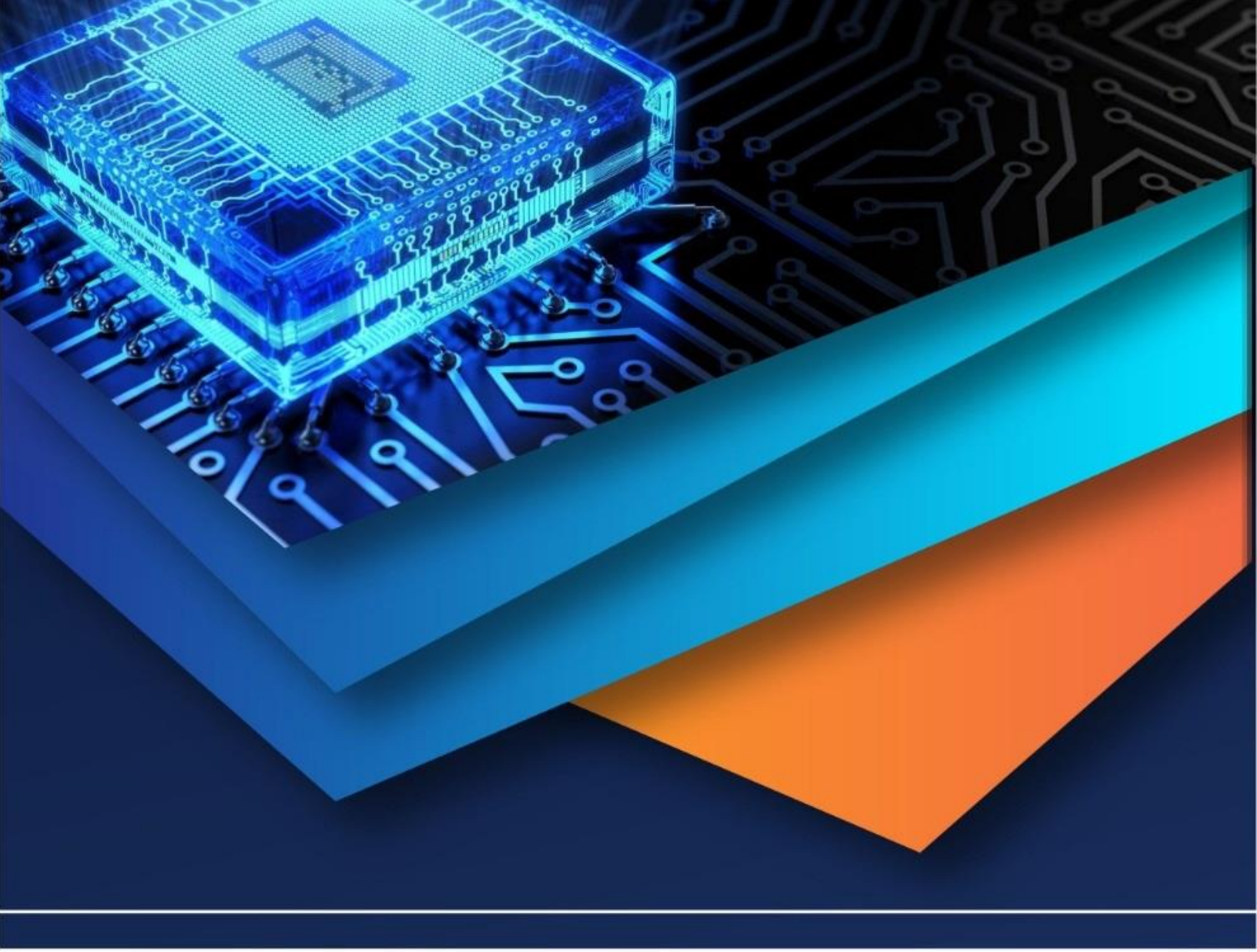

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