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Free Radical Scavenging Potential of Leaf of *Adhatoda Vasica* and *Lantana Camar* By DPPH Assay

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Abstract

Antioxidant have the capacity to prevent oxidative damage caused by free radical and can be used in anti-inflammatory and cardiovascular diseases. In the present study anti-oxidant activity of plant extracts of *Adhatoda visca* and *Lantana camara* were screened for antioxidant activities using in-vitro scavenging of free radicals by DPPH against ascorbic acid as standard antioxidant and leaf of methanolic, ethyl acetate, and aqueous fraction of these plants were subjected to antioxidant assay. The fractions showed dose dependent free radical scavenging property. Ethyl acetate and aqueous extracts of *Adhatoda visca* fraction were found to 75.99µg/ml, 79.16µg/ml, and 75.04µg/ml, respectively. The respective *Lantana camara* fraction showed 70.22 µg/ml, 80.92 µg/ml, and 70.50 µg/ml activities. On comparative basis leaf extracts of *Adhatoda visca* exhibit strong antioxidant.

Keywords

DPPH assay, Antioxidant, Ascorbic acid*, Adhatoda visca, Lantana camara.*

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INTRODUCTION

Medicinal plants have played a tremendous role in various system of medicine Worldwide. Traditionally, the World Health Organization (WHO) estimates that 4 billion people of the world population presently use plant-based medicine for their primary health care. The plants play a very important role in both developed and developing countries in the world for their valuable medication with minimal side effects than synthetic drugs. Economic commercialization and further globalization of plant products are gaining importance. In the present years, medicinal plants research has attracted a lot of attentions globally (Ahmed, 2007; Ansari, 2006). The large number of evidences has been accumulating to demonstrate that medicinal plant applications in various

complementary, traditional and alternate systems for treatment of various human disorders. From the nature numbers of modern drugs have been isolated from natural sources; many of them are use in traditional medicine. Therefore, ethanobotany seeks to provide a breather resource towards sustainable living. The natural antioxidants have acquired much importance in human health in recent times. Plants are potential sources of natural antioxidants that have immense therapeutic value. Many complex diseases can be treated and prevented with antioxidant-drugs. Many Indian medicinal plants are a rich source of antioxidants. The literature survey indicates that there are over 40 medicinal plants with antioxidant abilities at various levels of protection. The natural antioxidants have acquired much importance in human health in recent times. Plants are potential sources of natural antioxidants that have immense therapeutic value. Many complex diseases like atherosclerosis, stroke, diabetes and cancer can be treated and prevented with antioxidant-drugs (Devasagayam et al., 2004). The medicinal plants that show significant antioxidant activity include *Adhatoda visca* and *Lantana camara* were screened for antioxidant activities using *in-vitro* scavenging of free radicals by DPPH against ascorbic acid as standard antioxidant. Many other plant species have been investigated in the search for novel antioxidants (Buyukokuroglu 2001) but generally there is still a demand to find more information regarding the antioxidant potential of plant species as they are safe and also bioactive. Therefore, in recent years, considerable attention has been focused towards the identification of plants with antioxidant ability. Flavonoids and alkaloids are widely distributed secondary metabolites with antioxidant and antiradical properties. (Aqil et al, 2006). This paper reports the *in-vitro* antioxidant activity of selected medicinal plant extracts for potential application as anticancer agent.

2. MATERIALS AND METHODS

2.1 Chemicals

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals used were of analytical grade.

2.2 Collection of plant material

On the basis of ethnobotanical available literature and visual observation of plants that were relatively free from diseases and insect damages two plant species, *Adathoda visca, Lantana camara,* have been selected for the present study .The collected plant material were thoroughly washed and then dried under shade at 25± 2°C for about10 days. The dried plant samples were ground well into a fine powder in a mixer grinder. The powdered samples were then stored in air tight containers at room temperature.

2.3. DPPH scavenging activity

The free-radical scavenging capacity of different extracts of selected plants was evaluated with the DPPH stable radical, by the methodology described by Blois (1958). Briefly, 0.1mM alcoholic solution of DPPH was prepared and 2ml of this solution was added to 0.3ml of different extract concentrations (1-100 mg/ml) and allowed to react at room temperature. After 30min, the absorbance values were measured at 517nm against a suitable blank. DPPH radical scavenging by L-ascorbic acid (0.1M) was used as a standard. The radical scavenging activity (percent inhibition) was expressed as percentage of DPPH radical elimination calculated according to the following equation:

The percentage inhibition of $DPPH^{\cdot}$ in the reaction medium was calculated by comparing with the control.

$$\% \text{ inhibition} = A_{standard} - A_{sample} / A_{control} \times 100$$

RESULTS

DPPH assay has been extensively used for rapid screening antioxidant activity and is sensitive enough to detect active ingredients at low concentration. When DPPH radicals come across a free radical bearing substance, it would be scavenged and the absorbance decreases. Hence, DPPH radicals are widely used to investigate the scavenging activity of some natural compounds. In the present study, the methanol and ethanol extracts and aqueous extracts of *Adathoda visca, Lantana camera* are investigated for their antioxidant properties (Table 1-4).

Table 1- Antioxidant potential of alkaloids fraction of *Adathoda visca* leaf extracts.

Conc.of Extract/Standard (mg/mL)	Effect (%) for Ascorbic acid	Effect (%) for Ethanol extract	Effect (%) for Methanol extract	Effect (%) for Aqueous extract
100	96.41	75.17	81.07	71.32
90	96.32	75.14	80.08	71.21
80	96.34	74.02	79.06	70.13
70	95.24	72.06	76.08	68.06
60	94.25	72.01	76.00	67.08
50	94.12	69.10	70.30	65.09
40	87.31	66.05	69.60	64.08
30	67.87	61.10	66.02	60.00
20	49.02	56.00	62.10	58.08
10	30.00	40.56	51.08	46.10
1	15.00	20.06	28.07	21.06

(Given values are average of triplicate i.e. n=3)

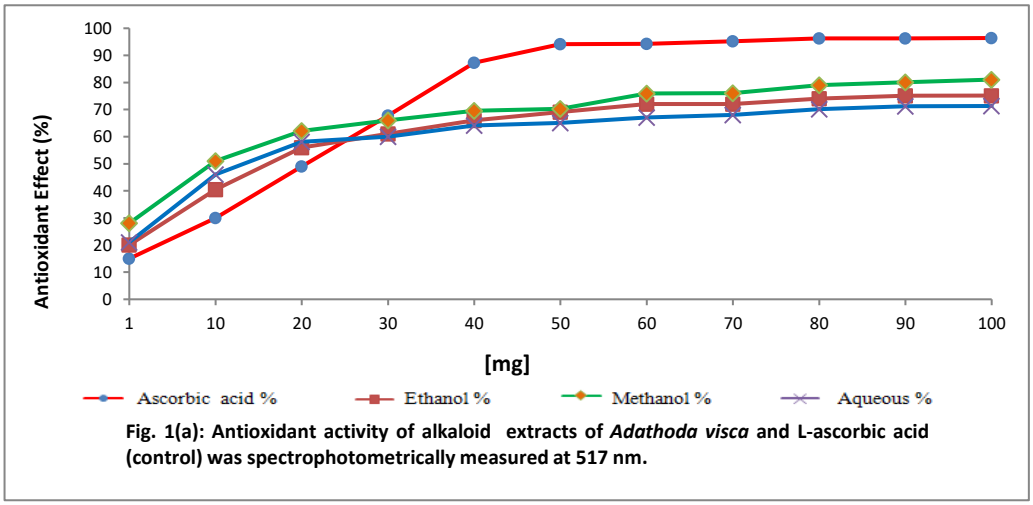

Fig. 1(a): Antioxidant activity of alkaloid extracts of *Adathoda visca* and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm.

Alkaloids extract of *A. visca* show DPPH reduction (methanol 81.07 %, ethyl alcohol 75.17 % and acetone 71.32 %) at 100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 81.07 % of *A. visca* (Fig.1(a).

Table 2- Antioxidant potential of alkaloids fraction of *Lantana camera* leaf extracts.

Conc.of Extract/Standard (mg/mL)	Effect (%) for Ascorbic acid	Effect (%) for Ethanol extract	Effect (%) for Methanol extract	Effect (%) for Aqueous extract
100	96.41	70.22	80.92	70.50
90	96.32	70.09	80.60	70.25
80	96.34	65.07	77.10	66.06
70	95.24	62.00	74.02	64.10
60	94.25	59.20	69.40	60.08
50	94.12	56.63	64.08	57.02
40	87.31	50.06	59.10	51.15
30	67.87	46.80	54.08	47.10
20	49.02	40.06	49.10	42.04
10	30.00	38.23	40.65	39.00
1	15.00	16.00	20.07	17.04

(Given values are average of triplicate i.e. n=3)

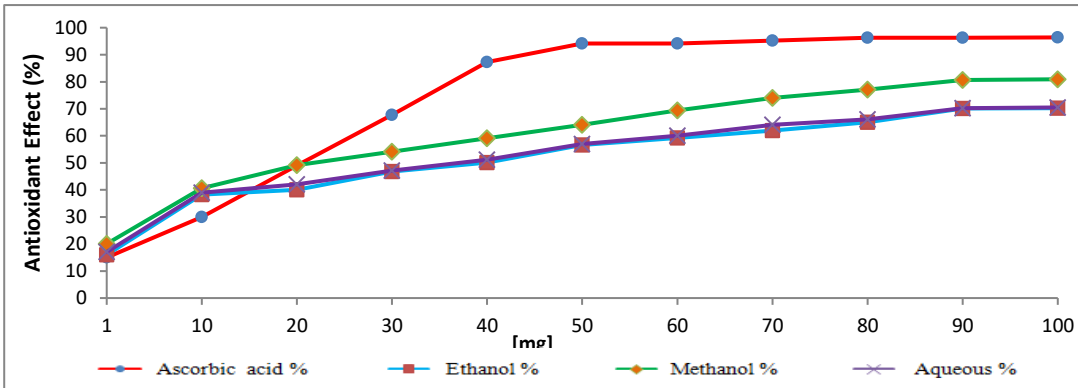

Fig. 1 (b): Antioxidant activity of Alkaloids extracts of Lantana camera and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm.

Alkaloids extract of *L. camera* show DPPH reduction (methanol 80.90 %, ethanol 70.22 % and aqueous 70.50 %) at 100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 80.90 % µg/ml of *Lantana camera* (Fig. 2 (b).

Table 3- Measurement of Antioxidant effects in Flavonoids fraction of *Adathoda visca* leaf extracts.

Conc.of Extract/Standard (mg/mL)	Antioxidant Effect (%) of Ascorbic acid	Antioxidant Effect (%) of Ethanol extract	Antioxidant Effect (%) of Methanol extract	Antioxidant Effect (%) of Aqueous extract
100	96.4	79.16	85.10	44.32
90	96.3	78.80	84.40	44.00
80	96.3	78.00	80.64	40.80
70	95.2	76.40	78.00	38.39
60	94.2	74.32	75.40	36.00
50	94.1	68.90	71.70	32.60
40	87.3	66.40	68.00	30.86
30	67.8	60.80	64.70	27.89
20	49.0	58.00	59.64	24.02
10	30.0	54.54	56.30	20.00
1	15.0	32.50	35.88	10.09

(Given values are average of triplicate i.e. n=3)

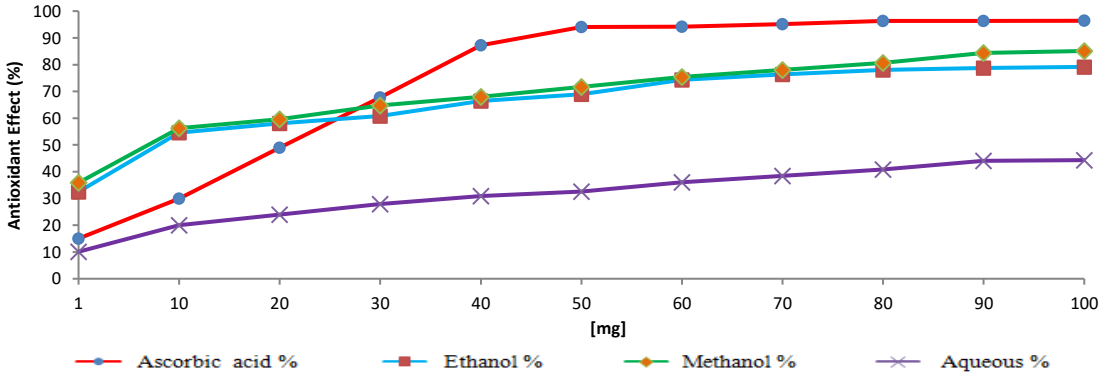

Fig. 2(a): Free radical scavenging activity of Flavonoids extracts of Adathoda visca and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm using the DPPH assay

Flavonoids extract of *A. visca* show DPPH reduction (methanol 85.10 %, ethanol 79.16 % and aquous 44.32 %) at 100 µg/ml. The maximum antioxidant effect was found in the methanol extract i.e 85.10 % µg/ml of *A. visca* (Fig.2 (a).

Table 4- Measurement of Antioxidant effects in Flavonoids fraction of *Lantana camera* leaf extracts.

Conc. of Extract/Standard (mg/mL)	Antioxidant Effect (%) of Ascorbic acid	Antioxidant Effect (%) of Ethanol extract	Antioxidant Effect (%) of Methanol extract	Antioxidant Effect (%) of Aqueous extract
100	96.4	68.47	80.90	37.72
90	96.3	68.00	80.02	37.20
80	96.3	66.50	78.40	34.66
70	95.2	65.08	76.50	30.89
60	94.2	60.30	72.55	30.10
50	94.1	56.90	68.90	28.40
40	87.3	54.23	64.76	26.33
30	67.8	48.00	58.00	21.87
20	49.0	38.40	56.65	18.66
10	30.0	30.22	46.00	12.00
1	15.0	15.06	28.10	09.00

(Given values are average of triplicate i.e. n=3)

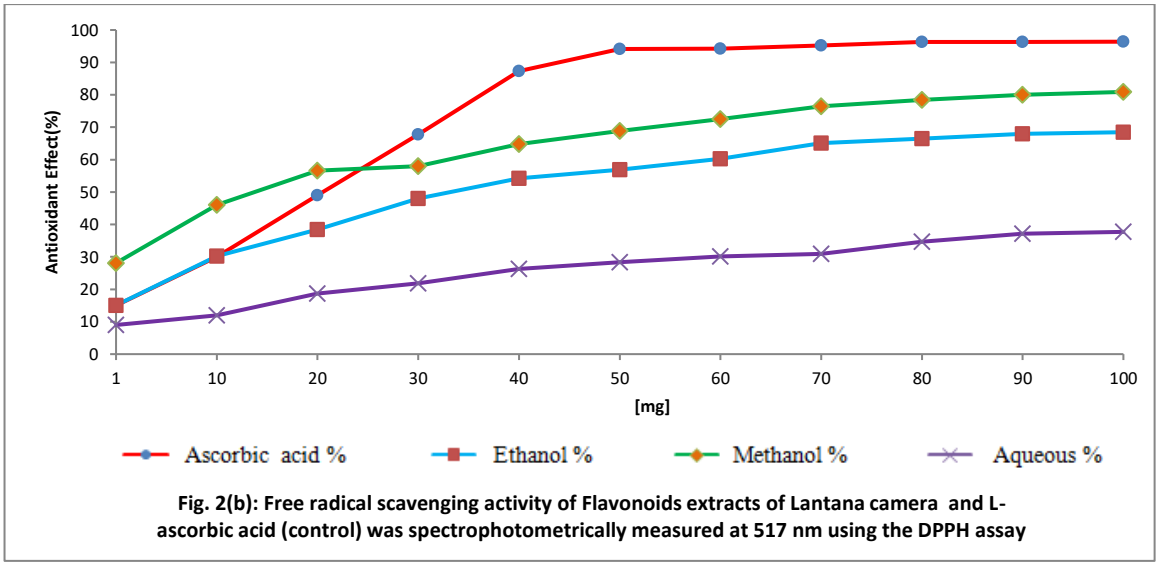

Fig. 2(b): Free radical scavenging activity of Flavonoids extracts of Lantana camera and L-ascorbic acid (control) was spectrophotometrically measured at 517 nm using the DPPH assay

Flavonoids extract of *L. camera* show DPPH reduction (methanol 80.90 %, ethanol 68.47 % and aqueous 37.72 %) at 100 µg/ml. The maximum anti-oxidant effect was found in the methanol extract i.e 80.90 % µg/ml of *L. camera* (Fig.2(b).

DISCUSSION

Antioxidant or free radical scavenging activity of extracts from these selected medicinal plants prepared in different solvents was investigated adopting DPPH model system. This study indicated that alkaloid and flavonoid fractions of the plant leaves of *Adathoda visca, Lantana camara* are endowed with antioxidant potential. Further the flavonoid fraction of the extract showed highest antioxidant activity than the alkaloid fraction of the extracts.

The results from the study show that flavonoid extract of *Adathoda visca* (85.10%) and *Lantana camara* (80.90%) have high potential as antioxidants. Our results compare with other medicinal plants; the ethanolic extracts of *Ocimum basillicum* leaf ranked first with an inhibition of free radical scavenging (96.18%). This is followed by *Alpinia calcarata* leaf (94.63 %). The plant extracts namely *Verbascum thapsus* leaf, *Jatropa gossipifolia* leaf, *Jatropa multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf, *Clitorria ternate* leaf and flower exhibited similar scavanging effects (Nunez et al,. 2002). The plant extracts showed low radical scavenging effects as seen with etanolic extracts of *Jatropha curcas* fruit, *Acorus calamus* leaf, *Strebilis aspera* leaf, *Passiflora edulis* fruit and *Sauropus androgynous* leaf. *Ocimum basillicum* leaf, *Alpina calcarata leaf*, *Jatropa multifida* flower, *Hyptis suaveolens* leaf, *Solanum indicum* leaf and *Clitorria ternate* leaf & flower possessed higher radical scavenging activity in both the solvent systems.

The antioxidant capacity of plant extract may be due to the hydrogen donating ability of phenols and flavonoids present in it (Nithya & Balakrishnan 2011).

In the present study among the three solvent extracts tested, the methanolic extracts exhibited maximum radical scavenging effect, followed by ethanol and aqueous extracts. The present investigation suggests that medicinal plants which possess good antioxidant potential are the best food supplements for the diseases associated with oxidative stress.

CONCLUSION

This study reveals that tested plant materials from *Adathoda visca***,** Lantana *camara* exhibit moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of natural antioxidants for pharmacological preparations.

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