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Original Article

# *In vitro* antioxidant studies of ethanol extract of roots of *Abutilon indicum*

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**Abstract:** The antioxidant activity of defatted ethanol extract of the roots of *Abutilon indicum* was studied for its free radical scavenging property on different *in vitro* antioxidant models like DPPH radical, Hydroxyl radical, Nitric oxide radical scavenging activity, Reducing power, Superoxide dismutase radical scavenging activity. The extract showed good free radical scavenging property in dose-dependent manner.

**Keywords:** *Abutilon indicum*, Free radical, Anti-oxidant

## Introduction

*Abutilon indicum* (F: Malvaceae) is found abundantly in the hotter parts of India, found as a weed in sub-Himalayan tracts, in wastelands throughout India, in tropical region of America, Malaysia, subtropical region and Ceylon (Sri Lanka). The root of the plant is traditionally used as demulcent, diuretic, in chest infection and urethritis[1]**.** Tribal people of Chittoor district, A.P. use *Abutilon indicum* (*A. indicum)* to treat such ailments where free radicals are involved. Eg: Hepatotoxicity, Asthma, Dropsy, etc. There were no reports on systematic study of the antioxidant activity of this plant. Therefore, the present study was carried out to screen the antioxidant activity of alcohol extract of the roots of *A. indicum*.

## Materials and Methods

### Chemicals:

Thiobarbituric acid (TBA), trichloroacetic acid (TCA), Sodium nitroprusside, N- napthylethylenediamine dihydrochloride all chemicals used are of high purity and were obtained from S.D. fine chemicals Ltd., India. 1-1-diphenyl-2-picrylhydrazyl (DPPH), Griess reagent, NBT (Nitroblue tetrazolium) was obtained from Sigma Chemicals.

### Plant material:

*Abutilon indicum* roots were collected from Tirupati, Chittor Dt., A.P. and authenticated by botanist Dr. K. Madhava chetty, Asst. Professor, Dept. of Botany, S.V. University, Tirupati and voucher specimen was deposited in S.V. University Botany Department, Tirupati.

### Preparation of Ethanol Extract:

The roots of the plant were allowed to dry under shade. The dried roots were powdered in a Wiley mill. 500gm of dry powder was extracted with petroleum ether (3 L, 60-80$^{o}$c) and refluxed for 3hrs, then filtered and subjected to distillation under reduced pressure. The procedure was repeated for three times. Marc was dried and extracted with ethanol (3 L) and it is refluxed for 3 hrs. The extract was filtered, procedure was repeated for three times and concentrated in *vacuum* to get the semi-solid (Yield: 5.9%) which was used for anti-oxidant studies.

### *In vitro* antioxidant activity:

The antioxidant activity of the ethanol extract of roots of *Abutilon indicum* was studied by using different *in vitro* antioxidant models like DPPH radical, nitric oxide radical scavenging activity, Superoxide dismutase radical, reducing power and Hydroxyl radical scavenging activity.

The antioxidant profile of Ethanol extract of roots of *Abutilon indicum* has been evaluated at concentrations of 50, 100, 200, 300, 400, and 500 µg/ ml. The percentage inhibition activity was recorded in a graded response.

$$\textbf{Percentage inhibition} = A_C - A_T / A_C \times 100$$

Where $A_C$ and $A_T$ are the absorbance values of the Control and of the Test sample(extract), respectively. A percentage inhibition versus concentration curve was plotted and the concentration of sample required for 50%

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inhibition was determined and expressed as **$IC_{50}$** value.

**DPPH radical scavenging activity:**

DPPH scavenging activity was measured by the Spectrophotometric method[2]. To an ethanol solution of DPPH (200µM), 0.05ml of extract dissolved in ethanol was added at different concentrations (50-500µg/ml). An equal amount of ethanol was added to the control. After 20min the decrease in absorbance of extract was read at 517nm and the percentage inhibition was calculated[3].

**Hydroxyl radical scavenging activity:**

Hydroxyl radical was generated by the $Fe^{3+}$-ascorbate-EDTA-$H_2O_2$ system (the Fenton reaction)[4]. The reaction mixture contained, in a final volume of 1 ml 2-deoxy-2-ribose (2.8 mM); $KH_2PO_4$-KOH buffer (20 mM, pH 7.4); $FeCl_3$ (100 µM); EDTA (100 µM); $H_2O_2$ (1.0 mM); Ascorbic acid (100 µM) and various concentrations (50-500 µg/ml) of the ethanol extract of plant. After incubation for 1 h at 37°C, 0.5 ml of the reaction mixture was added to 1 ml 2.8% TCA, 1 ml 1% aqueous TBA and the mixture was incubated at 90°C for 15 min to develop the pink chromogen. After cooling, the absorbance was measured at 532nm against an appropriate blank solution. All tests were performed in triplicate. Percentage inhibition was evaluated by comparing the extract and blank solutions.

**Scavenging of nitric oxide radical:**

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction[5-6]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentrations (50-500 µg/ml) of the ethanol extract dissolved in phosphate buffer (0.025M, pH: 7.4) and the tubes were incubated at $25^0$C for 5hrs. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5hrs, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess' reagent (1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed was read at 546nm[7]. The experiment was repeated in triplicate.

**Reducing power:**

The reducing power of Ethanol extract of *Abutilon indicum* was determined according to the standard method[8]. Different Concentrations of *A. indicum* extract was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and add potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%). The mixture was incubated at $50^0$C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. All the tests were performed in triplicate.

**Superoxidase dismutase radical scavenging activity:**

The Scavenging activity towards the superoxide radical ($O_2^{\cdot -}$) was measured in terms of inhibition of generation of $O_2^{\cdot -}$ by following alkaline DMSO method[9-10]. Potasssium superoxide and dry DMSO were allowed to stand in contact for 24hr and the solution was filtered immediately before use. Filtrate (200µl) was added to 2.8ml of an aqueous solution containing NBT (56 µM), EDTA (10µM) and potassium phosphate buffer (10mM). Ethanol extract (1ml) at various concentration (50-500µg/ml) were added, the absorbance was recorded at 560 nm against control.

**Statistical Analysis**:

All measurements were repeated three times. The values are expressed as Mean±Standard Deviation. The data was analyzed by using linear regression Analysis to calculate the $IC_{50}$ values.

## Results

**Inhibition of DPPH Radical:**

The extract was showed significant free radical scavenging activity and activity was dose dependent. The highest activity was observed at 500µg/ml i.e., 70.67 % (Extract) and 79.51 % (Ascorbic acid). The $IC_{50}$ value (the inhibitory concentration at which there is 50% reduction of free radical) of *Abutilon indicum* was found to be 210µg/ml.

**Hydroxyl radical scavenging:**

The effect of *Abutilon indicum* on hydroxyl radical and iron (II)-dependent deoxyribose damage was protected significantly at all concentrations. The percentage of inhibition of hydroxyl radical being 78.43% (500µg/ml) and The $IC_{50}$ value was found to be 260µg/ml.

**Table.1:** *In vitro* anti-oxidant activity of ethanol extract of roots of *Abutilon indicum*

| Concentration (µg/ml) | Percentage Inhibition | | | |
|---|---|---|---|---|
| | DPPH | Hydroxyl Radical | Nitric oxide | Superoxide Dismutase |
| 50 | 20.37 | - | 25.65 | 30.3 |
| 100 | 35.83 | 20.25 | 38.78 | 47.91 |
| 200 | 49.90 | 33.98 | 46.73 | 51.04 |
| 300 | 57.3 | 54.24 | 55.44 | 61.74 |
| 400 | 65.21 | 66.66 | 62.53 | 76.22 |
| 500 | 70.67 | 78.43 | 71.61 | 85.84 |
| Ascorbic acid (500 µg/ml) | 79.51 | 76.47 | 74.57 | 80.80 |
| $IC_{50}$ (µg/ml) | | | | |
| | 210 | 260 | 240 | 170 |

Values are reported as Mean ± Standard Deviation (n=3)

**Table.2:** Reducing Power of ethanol extract of roots of *Abutilon indicum*

| Concentration. (µg/ml) | Absorbance |
|---|---|
| 50 | 0.311 |
| 100 | 0.326 |
| 200 | 0.409 |
| 300 | 0.460 |
| 400 | 0.607 |
| 500 | 0.654 |
| Ascorbic acid (500 µg/ml) | 0.727 |

Values are reported as Mean ± Standard Deviation (n=3)

**Nitric oxide scavenging activity:**

The scavenging of nitric oxide by *Abutilon indicum* was concentration dependent. There was a potent inhibition of nitric oxide formation, with the maximum inhibition being at 500µg/ml i.e., 71.61%. The $IC_{50}$ value of the extract in this assay was found to be 240µg/ml.

**Reducing power activity:**

The reducing Power being 0.654 and 0.727 at 500 µg/ml for Abutilon *indicum* and Ascorbic acid respectively.

**Superoxide radical scavenging:**

The $IC_{50}$ values of the plant extract was found to be 170µg/ml. The maximum percentage inhibition of the plant extract was 85.84% (500µg/ml) whereas ascorbic acid exhibited 80.80% (500µg/ml). These data support the theory of free radicals scavenging having responsibility for the antioxidant action of the extract.

## Discussion

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules leading to various disease conditions, especially degenerative diseases and extensive lysis[11]. Many synthetic drugs protect against oxidative damage but they have adverse side effects. An alternative solution to the problem is to consume natural antioxidants from food supplements and traditional medicines [12-13]. Recent reports suggest that, many plants are rich in antioxidants [14-16] which may be used to treat various diseases where in free radicals were involved. *Abutilon indicum* is one such plant which has claims to treat various free radicals involving ailments such as hepatotoxicity, dropsy, throat infection and asthma etc [1]. On thorough literature survey it reveals that, there were no reports on *in vitro* antioxidant activity of *Abutilon indicum*. Hence present study is planned for *in vitro* antioxidant activity of roots of *Abutilon indicum*.

The ethanol extract of roots of *Abutilon indicum* was evaluated by using various *in-vitro* antioxidant models such as DPPH radical, hydroxyl radical, nitric oxide scavenging, reducing power, superoxide anion scavenging activities and the activity was compared with the standard i.e., Ascorbic acid.

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by Antioxidants [18]. The study showed that the extract have the proton-donating ability and could serve as free radical inhibitor or scavenger.

Hydrogen peroxide is a weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. It can cross cell membranes rapidly, once inside the cell, it can probably react with $Fe^{2+}$ and possibly $Cu^{2+}$ ions to form hydroxyl radicals and this may be the origin of many of its toxic effects.

Hydroxyl radicals are produced by Fenton type reactions; in which transition metals (e.g. iron) reduce hydrogen peroxide. Reducing agents such as ascorbic acid can accelerate OH formation by reducing [$Fe^{3+}$] ions to [$Fe^{2+}$][4]. In the deoxyribose assay a mixture of [$Fe^{3+}$]-EDTA, [$H_2O_2$] and ascorbic

acid generates hydroxyl radical which can be detected by their ability to degrade the sugar deoxyribose into fragments[19]. If the resulting complex mixture of products is heated under acid conditions, malonaldehyde is formed and may be detected by its ability to react with thiobarbituric acid to form a pink Chromogen.

The effect of *Abutilon indicum* and Ascorbic acid on the inhibition of free radical-mediated deoxyribose damage was assessed by means of iron (II)-dependent DNA damage assay. The extract showed significant effect as comparable with ascorbic acid.

Nitric Oxide (NO) is an important modulator of physiological and pathological function in the cardiovascular, neuronal and immune systems. Nitric oxide was generated from nitroprusside and the nitrate formed was measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide [5-6], which interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide.

The extract inhibited nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The ethanol extract of roots of *Abutilon indicum* significantly inhibited the production of nitric oxide.

Reducing power is to measure the reductive ability of antioxidant and it is evaluated by the transformation of Fe (III) to Fe (II), by donating an electron in the presence of the sample extracts [20]. The reducing power is increased with an increasing the concentration of extract. In present study the extract reduced the generation of free radicals, probably due to a combination of its abilities of free radicals scavenging, binding to Fe. The ethanol extract of roots of *Abutilon indicum* had shown good reducing power that was comparable with Ascorbic acid.

The superoxide radicals generated from dissolved oxygen by PMS-NADH coupling can be measured by their ability to reduce NBT. The decrease in absorbance at 560 nm with the plant extract and the reference compound, Ascorbic acid indicates their abilities to quench superoxide radicals in the reaction mixture.

Capability of the *Abutilon indicum* extracts to interact with $O_2^{\cdot}$ radicals was measured as a function of its inhibition effect on the NBT reduction caused by these radicals. It is evident that the *Abutilon indicum* extract's capacity to react directly with $O_2^{\cdot}$ radicals depends on its concentration.

## Conclusion

In conclusion, the results of the present study suggest that tested plant material exhibited potent antioxidant activity.

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