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# PRELIMINARY PHYTOCHEMICAL INVESTIGATION, *IN-VITRO* ANTIOXIDANT, *IN-VITRO* α-AMYLASE INHIBITION, *IN-VITRO* AND *IN-VIVO* ANTICATARACT ACTIVITY OF ETHYLACETATE ROOT EXTRACT OF *ABUTILON INDICUM*

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**Keywords:**

Free radicals, *Abutilon indicum*, Ascorbic acid, Acarbose, α-Amylase inhibition, Anticataract

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**ABSTRACT:** The present study aims to evaluate the *in-vitro* antioxidant, *in-vitro* α-amylase inhibition, *in-vitro* and *in-vivo* anti-cataract potential of ethyl acetate root extract of *Abutilon indicum*. Antidiabetic activity was evaluated against alpha-amylase by spectrophotometric assays. DPPH determined antioxidant activity, Reducing power assay, Phosphomolybdenum antioxidant assay, Nitric oxide-scavenging activity, Hydrogen peroxide scavenging, and Hydroxyl radical scavenging assay. Different extract concentrations were made using dimethyl sulfoxide (DMSO) and subjected to an α-amylase inhibitory assay. The *in-vitro* anticataract potential of *Abutilon indicum* was determined using glucose-induced cataractous goat eye lens. In *the in-vivo* group, cataract was induced in rats by 30% galactose diet alone (control) or with the addition of *Abutilon indicum* (treated group). The results indicate the dose-dependent *in-vitro* antioxidant activity against DPPH, Reducing power assay, Phosphomolybdenum antioxidant assay, Nitric oxide-scavenging activity, Hydrogen peroxide scavenging, and Hydroxyl radical scavenging comparable with that of standard Ascorbic acid and also appreciable α-amylase inhibitory activity with $IC_{50}$ values comparable with that of standard Acarbose. An *in-vitro* study was conducted, which reported that the lens group treated with the plant extract (500µg/ml) exhibited a reduction in opacity compared to the lens in the negative control. The study of the anti-cataract potential of *Abutilon indicum* exhibited an increase in total protein content, aldose reductase inhibition activity and a decrease in the malondialdehyde level compared to the negative control. *Abutilon indicum* can delay the onset and progression of cataracts in an experimental rat model of Glucose-induced cataracts *in-vitro* and galactose-induced cataracts *in-vivo*.

**INTRODUCTION:** Diabetes mellitus is an endocrine disorder characterized by increased glucose levels. It mainly affects humans due to defects in insulin secretion or resistance [1].

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Pancreatic alpha-amylase is the key enzyme in the small intestine. These enzymes play a major role in the digestion of starch yielding glucose and maltose, leading to increased postprandial glucose levels [2].

Hence, reducing the starch digestion rate by inhibiting enzymes such as alpha-amylase is the best way to manage diabetes [3]. Oxidative stress induced by free radicals is also one of the causative factors for diabetes. Antioxidant compounds play an important role in free radical scavenging and

controlling diseases related to oxidative stress [4]. During the last decade, considerable attention has been focused on the involvement of Reactive Oxygen Species (ROS) in various diseases. The generation of free radicals causes cumulative damage of DNA, proteins; lipids led to oxidative stress. This oxidative stress has been suggested to cause aging and various human diseases like cancer, hepatic disorders, and diabetes [5]. Therefore, there has been a growing interest in finding novel antioxidants to meet pharmaceutical industries' requirements [6]. A cataract is the major cause of blindness, responsible for 50% of the global incidence [7]. Pharmacological intervention that prevents or slows the progression of cataractogenesis has a significant health impact. Our earlier studies screened natural antioxidants and herbal drugs and reported their potential anticataract activity [8].

*Abutilon indicum* is a medicinal plant belonging to the family Malvaceae. It is commonly called Thuthi / Atibala. In India, it is distributed throughout the hotter parts and used traditionally in various fields of medicine to treat diseases like jaundice, leprosy, diabetes, ulcer *etc*. [9]. From ancient times, this plant has been used in ayurvedic medicine with greater benefits [10]. The various parts of the plant, such as seeds, roots, and leaves, are reported to possess various medicinal benefits in ethnobotanical surveys conducted by ethnobotanists and in traditional systems of medicine such as Ayurveda. The root is cylindrical and nearly 1.4 -1.8 cm in diameter with a smooth surface and yellow in colour, having fragrance with salt like taste [11]. In Vedic periods, the roots of the *Abutilon indicum* were used as demulcent, analgesic, aphrodisiac, to remove poison, heart problems, eye diseases, and uterine disorders [12]. So far much pharmacological work has not been carried out on *Abutilon indicum* root. So, the present study was undertaken to evaluate the antioxidant, α-amylase inhibition, and anti-cataract activity.

## MATERIALS AND METHODS:

**Plant Material:** The roots of *Abutilon indicum* were collected near Peddapuram, Andhra Pradesh, India country. The plant was authenticated by Dr. T. Raghuram, Taxonomist, Maharani College, Peddapuram and the voucher specimen number given is 22125.

**Preparation of Extract [13]:** The freshly collected roots of *Abutilon indicum* were washed with water to remove dirt and sand particles and dried under shade for 40 days. They were grounded into powder using a mechanical grinder. The powder was extracted with 95% ethanol and ethyl acetate for 3 days, followed by hot percolation for 3 hours. Then it was filtered and distilled at 80°C. Then it was transferred into the empty china dish, evaporated to get an ethyl acetate extract, and kept in anhydrous calcium chloride containing desiccators.

**Phytochemical Screening:** In preliminary phytochemical testing, the ethyl acetate extracts of *Abutilon indicum* root extract were performed to test the presence of the secondary metabolites such as flavonoids, alkaloids, tannins, phenolic compounds, saponins, fixed oils, and fats [14].

### *In-vitro* Antioxidant Activity:

**DPPH Free Radical Scavenging Activity:** The free radical scavenging activity was followed by the DPPH method [15]. 0.1 mM solution of DPPH in methanol was prepared, and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.05, 0.1, 0.3, and 0.5 mg/ml). The absorbance was measured later after the completion of 30 minutes at 517 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (0.05, 0.1, 0.3 and 0.5 mg/ml) was used as standard. The experiment was repeated triplicate. The percentage inhibition was calculated using the following equation:

$$\text{DPPH (\%)} = (A_o - A_1) / A_o \times 100$$

Where, $A_o$ is the control reaction absorbance (containing all reagents except the sample extract), and $A_1$ sample extract absorbance. Ascorbic acid was used as standard.

**Phosphomolybdenum Antioxidant Assay:** The antioxidant activity of *Abutilon indicum* root extract was evaluated by the phosphomolybdenum method according to procedure [16]. The assay is based on Mo (VI)–Mo (V) reduction by the extract and at acid pH leads to the formation of a green phosphate/Mo (V) complex. 0.3 ml of extracts (0.05,0.1,0.3 and 0.5 mg/ml ) were combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium

molybdate). The reaction solution was incubated at 95°C for 90 min. The absorbance of the solution was measured at 695 nm.

**Nitric Oxide Generation and Assay of Nitric Oxide Scavenging:** Sodium nitroprusside in an aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated by Greiss reagent. Nitric oxide scavengers compete with oxygen, reducing nitric oxide production [17]. Sodium nitroprusside (5 mM) in phosphate-buffered saline was mixed with different concentrations of the extracts (0.05, 0.1, 0.3, and 0.5 mg/ml) dissolved in the suitable solvent systems and incubated at 25 °C for 150 min. The samples above were reacted with Greiss reagent (1% sulphanilamide, 2% $H_3PO_4$, and 0.1% naphthyl ethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamme was read at 546 nm and referred to the absorbance of standard solutions of ascorbic acid was treated in the same way with Griess reagent. The formula to calculate the percentage inhibition was

$$\text{Nitric oxide Scavenged (\%)} = (A_o - A_1) / A_o \times 100$$

Where, $A_o$ is the absorbance of the control reaction (containing all reagents except the sample extract), and $A_1$ is the absorbance of the sample extract. Ascorbic acid were used as positive control.

**Reducing Power Method:** Electron donating activity is indicated by Fe (III) reduction, which is an important mechanism of phenolic antioxidant action. Different concentration of *Abutilon indicum* root extract (0.05,0.1,0.3 and 0.5 mg/ml) extract in 1ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) & potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50 º 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. Ascorbic acid was used as standard. Increased absorbance of the reaction mixture indicates an increase in reducing power.

**Hydroxyl Radical Scavenging Assay:** The scavenging ability of the five sample extracts on hydroxyl radicals was determined according to the method described by [18] with some modifications. Briefly, individual sample extracts (1 mL) at different concentrations (0.05,0.1,0.3 and 0.5 mg/ml) was added to the reagent containing 1 mL 1.5 mM $FeSO_4$, 0.7 mL 6 mM $H_2O_2$ and 0.3 mL 20 mM sodium salicylate. After incubation for 1 h at 37°C, the absorbance of the reaction mixture was read at 562 nm. The formula to calculate the percentage inhibition was

$$\text{Scavenging ability on hydroxyl radicals (\%)} = (A_o - A_1) / A_o \times 100$$

Where, $A_o$ is the control reaction absorbance (containing all reagents except the sample extract), and $A_1$ sample extract absorbance. Ascorbic acid was used as standard.

**Scavenging of Hydrogen Peroxide:** The ability of the extracts to scavenge hydrogen peroxide was determined. Hydrogen peroxide (40 mM) solution was prepared in phosphate buffer (pH 7.4). The hydrogen peroxide concentration was determined by absorption at 230 nm. Extracts (0.05, 0.1, 0.3 and 0.5 mg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The formula to calculate the percentage inhibition was

$$\text{Scavenging ability on hydroxyl radicals (\%)} = (A_o - A_1) / A_o \times 100$$

Where, $A_o$ is the control reaction absorbance (containing all reagents except the sample extract), and $A_1$ sample extract absorbance. Ascorbic acid was used as standard.

***In-vitro* Alpha Amylase Inhibition Activity:** The α-amylase inhibition assay was performed using DNSA method. The *Abutilon indicum* root extract was dissolved in a minimum amount of 10% DMSO and was further dissolved in buffer (($Na_2HPO_4$ /$NaH_2PO_4$ (0.02 M), NaCl (0.006 M) at pH 6.9) to give various concentrations. A volume of 200 µl of α-amylase solution (2 units/ml) was mixed with 200 µl of the extract and was incubated for 10 min at 30 °C. After that 200 µl of the starch

solution (1% in water (w/v)) was added to each tube and incubated for 3 min. The reaction was terminated by adding 200 µl DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 ml of 96 mM of 3,5-dinitrosalicylic acid solution) and was boiled for 10 min in a water bath at 85–90 °C. The mixture was cooled to ambient temperature and was diluted with 5 ml of distilled water, and the absorbance was measured at 540 nm using a UV-Visible spectrophotometer. The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µl of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration without the enzyme solution. Acarbose was used as a standard. The α-amylase inhibitory activity was expressed as percent inhibition and was calculated using the equation given below:

% α amylase inhibition = 100 × Abs 100% control – Abs Sample / Abs 100% Control

***Invitro* Anticataract Activity:**

**Collection of Goat Eye Balls:** The anticataract potential of the plant extract was studied *in-vitro* using goat eye lenses in glucose-induced cataractogenesis. Goat eyeballs were obtained from the slaughterhouse at Peddapuram immediately after slaughter and transported to the laboratory at 0-4°C.

**Preparation of Lens Culture:** The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl: 140 mM, KCl: 5 mM, MgCl2: 2 mM, NaHCO3: 0.5 mM, NaH(PO4)2: 0.5 mM, CaCl2: 0.4 mM, and Glucose: 5.5 mM) at room temperature and pH 7.8 for 72 hours. Penicillin G 32 mg% and Streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose 55 mM at the concentration was used for cataract induction.

**Experimental Design:**

**Group I:** Normal lens + glucose 5.5 mM (Normal control).

**Group II:** Lens + Glucose 55 mM (Negative control).

**Group III:** Lens + Glucose 55 mM + *Abutilon indicum* root extract (500 µg/ml).

**Group IV:** Lens + Glucose 55 mM + Standard drug Enalapril (10 ng/ml).

**Photographic Evaluation of Lens Opacity:** After incubation of 72 hrs, the opacity of lenses was observed, and photographs of lenses were captured on the wire meshes with posterior surface touching the mesh, and the pattern of mesh was observed as a measure of lens opacity through the lens.

**Preparation of Lens Homogenate:** Lenses were homogenized in Tris buffer (0.23 M pH 7.8) and 0.25 x 10-3 M EDTA. The homogenate was adjusted to 10% w/v. The homogenate was centrifuged at 10,000 rpm

**Study of Anticataract Potential of the Plant Extract:** The anticataract potential of the plant extracts was determined. The following biochemical parameters were analyzed.

**Estimation of Total Protein Content:** To lens homogenate of 0.1 ml, 4.0 ml of alkaline copper solution was added and allowed to stand for 10 min. Then, 0.4 ml of phenol reagent was added rapidly, mixed quickly, and incubated at room temperature for 30 min for color development. Reading was taken against a blank prepared with distilled water at 610 nm in a UV-visible spectrophotometer. The protein content was calculated from a standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue.

**Estimation of Malondialdehyde (MDA):** Lenses were homogenized in10% (w/v) 0.1 M Tris–HCl buffer (pH 7.5). One milliliter of the homogenate was combined with 2 ml of TCA–TBA–HCl reagent, 15% trichloroacetic acid (TCA), and 0.375% thiobarbituric acid (TBA) in 0.25 N HCl and boiled for 15 min.

The precipitate was removed after cooling by centrifugation at 1000 rpm for 10 min, and the absorbance of the sample was read at 535 nm against a blank without tissue homogenate. The values are expressed as MDA/min/mg lens protein.

**Determination of Aldose Reductase (AR) Activity:** AR activity was assayed according to the modified protocol described by Rajesh [20]. The assay mixture in 1 ml contained 0.7 ml phosphate

buffer (0.067 M), 0.1 ml of NADPH (25×10-5), 0.1 ml of lens supernatant, 0.1 ml of D L-glyceraldehydes (substrate) (5×10-4 M). Appropriate reference blanks were employed for corrections except the substrate, D L-glyceraldehydes.

The addition of substrate started the enzymatic reaction, and the absorbance was recorded in UVspectrophotometer at 340 nm for at least 3 min at 30-sec intervals. AR activity was expressed as Δ OD /min/mg protein, and the % inhibition activity was found using the following formula:

AR inhibition activity (%) = A340nm (Control) – A340nm (Sample) / A340nm (Control)

Where, A340nm (Control) is the absorbance of the control at 340nm, A 340 nm (Sample) is the absorbance of the plant sample at 340nm.

**Galactose Cataract *In-vivo* Model [19]:** *In-vivo* Wistar rats of either sex weighing 250g was divided into control and treated groups (n =6). 300g/L galactose was fed to all group's *ad libitum*-induced cataracts. Seven days before the start of the galactose diet, 50 and 100mg/kg body weight doses of *Abutilon indicum* extract in distilled water (as a vehicle) were given orally once a day to the treated group and continued till the end of the experiment. In the control group, only distilled water and the galactose diet were given. The eyes were examined through a slit lamp after dilating the rat pupil with 10g/L tropicamide. The stages of cataracts were graded according to Sippel's classification.

**Statistical Analysis:** Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple tests. Results are expressed as Mean±SEM for five rats in each group. Differences among groups were considered significant at P<0.001 level.

## RESULTS:

**Preliminary Phytochemical Screening:** The ethyl acetate extract was light brown. The preliminary phytochemical analysis of ethyl acetate extract of *Abutilon indicum* root consists of secondary metabolites like alkaloids, tannins, and Triterpenoids.

**TABLE 1: *IN-VITRO* ANTIOXIDANT POTENTIAL OF ETHYL ACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ASCORBIC ACID BY PHOSPHOMOLYBDATE AND REDUCING POWER ASSAY**

| Conc. (ug/ml) | Phosphomolybdate (Abs) | | Reducing Power (Abs) | |
|---|---|---|---|---|
| | Eth Acetate. Ext | STD | Eth Acetate. Ext | STD |
| 50 | 0.06±0.23 | 0.181±0.32 | 0.045±0.14 | 0.052±0.25 |
| 100 | 0.08±0.32 | 0.381±0.18 | 0.225±0.15 | 0.129±0.15 |
| 300 | 0.103±0.16 | 0.621±0.23 | 0.290±0.36 | 0.352±0.36 |
| 500 | 0.332±0.25 | 0.973±0.36 | 0.386±0.25 | 0.485±0.12 |

All the values are expressed as Mean ± SEM, n= 3; *P< 0.001 when compared with standard values.

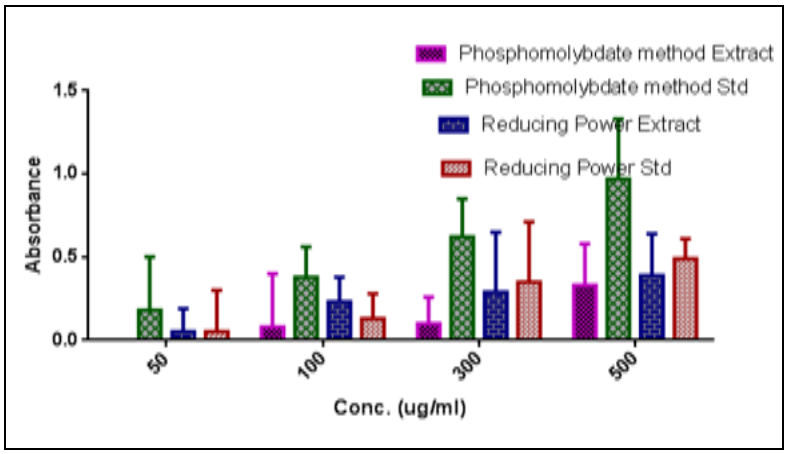


**FIG. 1: *IN-VITRO* ANTIOXIDANT POTENTIAL OF ETHYLACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ASCORBIC ACID BY.** Kinoshita JH, Kador P, Catiles M. Aldose reductase in diabetic cataracts. Jama. 1981 Jul 17; 246(3):257-61.

### *In-vitro* Antioxidant Activity:

**TABLE 2: *IN-VITRO* ANTIOXIDANT POTENTIAL OF ETHYL ACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ASCORBIC ACID AGAINST DPPH, NITRIC OXIDE, HYDROXYL, $H_2O_2$ RADICALS**

| Conc. ug/ml | DPPH (%) | IC50 ug/ml | | IC50 ug/ml | Nitric oxide (%) | IC50 ug/ml | | IC50 ug/ml | Hydroxyl (%) | IC50 ug/ml | | IC50 ug/ml | $H_2O_2$ (%) | IC50 ug/ml | | IC50 ug/ml |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eth Acetate. Ext | 87.09 | STD | 33.11 | Eth Acetate. Ext | 24.5 | STD | 7.94 | Eth Acetate. Ext | 97.7 | STD | 24.54 | Eth Acetate. Ext | 81.28 | STD | 40.7 |
| 50 | 41.66 ±0.32 | | 55.36 ±0.22 | | 52.71± 0.35 | | 58.45± 0.2 3 | | 38.51±0.0 6 | | 69.47 ±0.32 | | 43.84± 0.25 | | 51.22± 0.32 | |
| 100 | 52.32± 0.25 | | 60.32 ±0.27 | | 63.73± 0.18 | | 70.07 ±0.22 | | 47.91±0.0 6 | | 82.55 ±0.26 | | 52.48± 0.29 | | 60.08± 0.36 | |
| 300 | 65.82± 0.36 | | 70.85 ±0.13 | | 70.45± 0.13 | | 71.76± 0.32 | | 73.12±0.1 5 | | 83.46 ±0.12 | | 65.04± 0.15 | | 65.15± 0.19 | |
| 500 | 80.72± 0.69* | | 80.32 ±0.12 | | 78.29± 0.13* | | 74.84± 0.13 | | 80.62±0.0 8* | | 87.65 ±0.16 | | 70.77± 0.25* | | 75.19± 0.18 | |

All the values are expressed as Mean±SEM, n= 3; * P< 0.001 when compared with standard values.

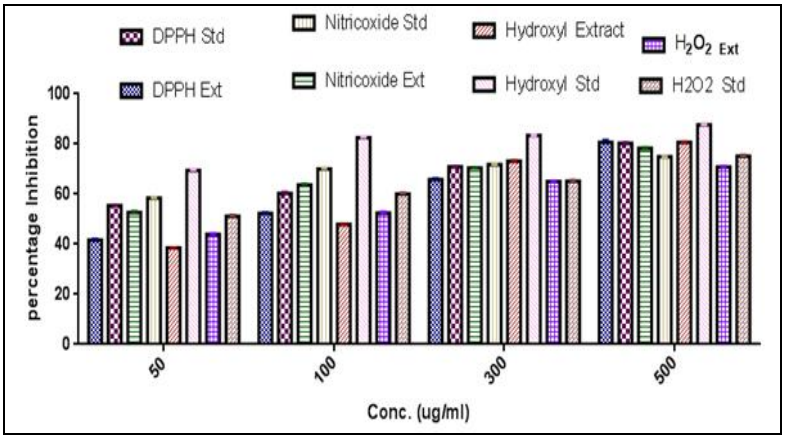


**FIG. 2: *IN-VITRO* ANTIOXIDANT POTENTIAL OF ETHYL ACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ASCORBIC ACID AGAINST DPPH, NITRIC OXIDE, HYDROXYL, $H_2O_2$ RADICALS**

## *In-vitro* Alpha Amylase Inhibition:

**TABLE 3: *IN-VITRO* ALPHA-AMYLASE INHIBITION OF ETHYL ACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ACARBOSE**

| Conc. (ug/ml) | Eth Acetate. Ext | IC50 (ug/ml) | Std | IC50 (ug/ml) |
|---|---|---|---|---|
| 50 | 50.71±0.001 | 44.77 | 56.62±0.17 | 22.3 |
| 100 | 62.22±0.005 | | 76.32±0.18 | |
| 300 | 75.41± 0.004 | | 88.78±0.012 | |
| 500 | 83.74± 0.007 | | 89.72±0.08 | |

All the values are expressed as Mean ± SEM, n=3

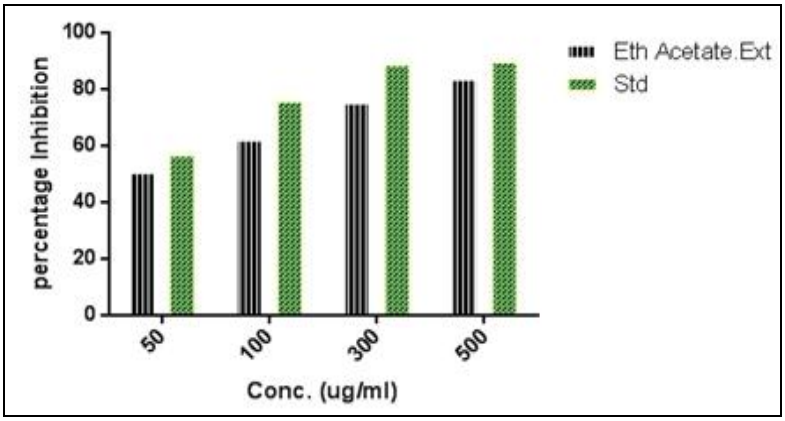


**FIG. 3: *IN-VITRO* ALPHA-AMYLASE INHIBITION OF ETHYL ACETATE ROOT EXTRACT OF *ABUTILON INDICUM* AND ACARBOSE**

## *In-vitro* Anticataract Activity:

## Photographic Evaluation of Lens Opacity:


**FIG. 4: PHOTOGRAPHIC EVALUATION OF THE LENS OPACITY**

**TABLE 4: EFFECT OF THE VARIOUS TREATMENT GROUPS ON LENS PROTEIN, MDA AND AR-INHIBITION ACTIVITY**

| Groups | Protein (mg/ml) | MDA (MDA/ min/ mg lens protein) | AR–Inhibition Activity (%) |
|---|---|---|---|
| Group I | 16.6 ± 0.316 | 0.0006 ± 0.0023 | 97.28 ± 0.0032 |
| Group II | 2.2 ± 0.162 | 0.0026 ± 0.0032 | 65.22 ± 0.0054 |
| Group III | 11.8 ± 0.235* | 0.0018 ± 0.0225* | 82.43 ± 0.0063* |
| Group IV | 12.8 ± 0.224 | 0.0008 ± 0.0153 | 90.40 ± 0.0041 |

All the values are expressed as Mean ± SEM, n=3; * P< 0.001 when compared with standard values.

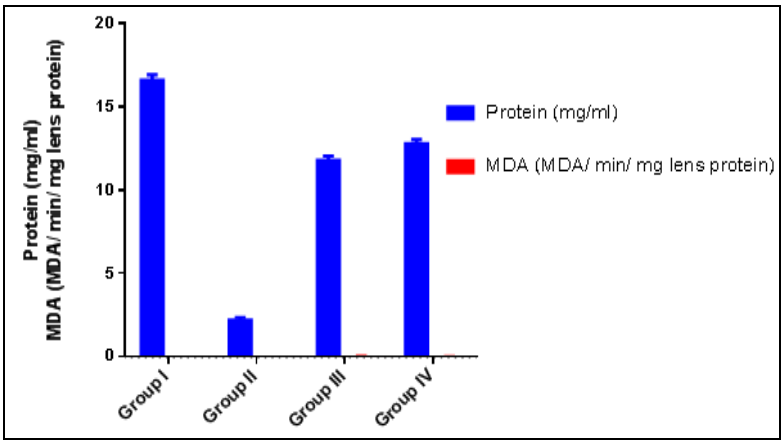


**FIG. 5: EFFECT OF THE VARIOUS TREATMENT GROUPS ON LENS PROTEIN AND MDA**

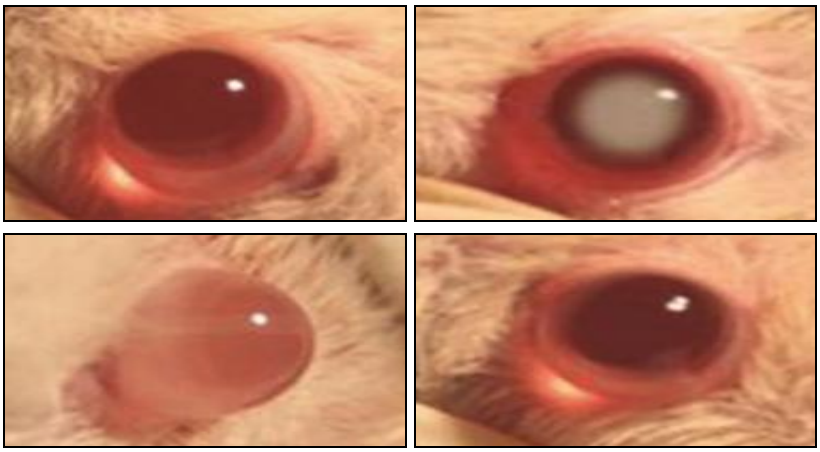

**FIG. 6: PHOTOGRAPHS OF LENS IN GALACTOSE CATARACT *IN-VIVO* MODEL. A) NORMAL CONTROL B) NEGATIVE CONTROL C & D): EYTHYLACETATE EXTRACT 50MG/KG & 100MG/KG**

**DISCUSSION:** DPPH assay has been widely used to provide basic information on the antioxidant ability of extracts from the plant, food materials, or single compounds. This method has shown to be

rapid and simple available [21]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability [22] and is a useful reagent for investigating the free radical scavenging activities of compounds [23]. DPPH radical is a stable free radical 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 induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants because the reaction between antioxidant molecules and the radical, progresses, which results in the scavenging of the radical by hydrogen donation.

These results indicated that ethyl acetate root extract of *Abutilon indicum* has a noticeable effect of scavenging free radicals, evident from **Table 2, Fig. 2**. It was reported that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biological basis of chronic condition [24]. The root extract of *Abutilon indicum* reacts with free radicals, which are the major initiator of the autoxidation chain of fat, thereby terminating the chain reaction [25]. Thus, Root extract of Abutilon indicum is a free radical inhibitor or scavenger, as well as a primary antioxidant that reacts with free radicals, which may limit free radical damage occurring in the human body. DPPH radical scavenging activity of Root extract of *Abutilon indicum* was similar to that of standard Ascorbic acid

The reducing power assay measures the electron-donating ability of antioxidants using the potassium ferricyanide reduction method. Antioxidants reduce the ferric ion/ferricyanide complex to the ferrous form, the Perl's Prussian blue complex [26]. A compound's reducing capacity may be a significant indicator of its potential antioxidant activity [27]. The antioxidant activity of Aqueous and Ethanolic root extract of *Asparagus racemosus* and Ascorbic acid have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [28]. The reducing capacity of ethylacetate root extract of *Abutilon indicum* and Ascorbic acid indicates their potential antioxidant activity **Table 1, Fig. 1**. OH, radical is the most reactive free radical in biological systems. It can be formed from superoxide anion and hydrogen peroxide in the presence of metal ions, such as copper and iron. Hydroxyl radical has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. For example, OH-radicals react with lipid, polypeptides, proteins, and DNA, especially thiamine and guanosine. This radical has the capacity to conjugate with nucleotides in DNA, cause strand breakage, and lead to carcinogenesis, mutagenesis and cytotoxicity [29]. The highly reactive OH radicals can cause oxidative damage to DNA, lipids, and proteins [30]. As is the case for many other free radicals, OH radicals can be neutralized if it is provided with a hydrogen atom. The results in **Table 2, Fig. 2** indicate that Ethylacetate root extract of *Abutilon indicum* had strong hydroxyl radical scavenging activity similar to Ascorbic acid.

$H_2O_2$ is highly important because of its ability to penetrate biological membranes. $H_2O_2$ is not very reactive, but it can sometimes be toxic to cells because it may give rise to hydroxyl radical in the cells [31]. Thus, removing H2O2 is very important for the protection of living systems. The results in **Table 2, Fig. 2** indicate that Ethylacetate root extract of Abutilon indicum had strong hydrogen peroxide radical scavenging activity similar to Ascorbic acid.

The nitric oxide radical generated from sodium nitroprusside at physiological pH was inhibited by Ethylacetate root extract of *Abutilon indicum* and Ascorbic acid. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide [32] which interacts with oxygen to produce nitrite ions that can be estimated using Greiss reagent. Scavengers of nitric oxide compete with oxygen leading to reduced nitric oxide production. Ethylacetate root extract of *Abutilon indicum* had comparably more nitric oxide radical scavenging activity than Ascorbic acid **Table 1, Fig. 1**. The total antioxidant capacity of Ethylacetate root extract of *Abutilon indicum* was determined by phosphomolybdenum assay and the highest absorbance was recorded at 0.5mg/ml **Table 1, Fig. 1.** The antioxidant capacity of the

Ethylacetate root extract of *Abutilon indicum* was measured by phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/ Mo (V) compounds with maximum absorption at 695 nm. The antioxidant capacity of extracts was found to increase with the increase in concentration.

The α-amylase inhibitory studies carried out indicated that the Ethylacetate root extract of Abutilon indicum had significant inhibitory potentials. The $IC_{50}$ value of ethylacetate extract is almost similar to that of Acarbose, a widely used and marketed anti-diabetic drug, and the results are displayed in **Table 3**. These α- amylase inhibitors are also called starch blockers as they prevent or slow the absorption of starch into the body mainly by blocking the hydrolysis of 1,4-glycosidic linkages of starch and other oligosaccharides into maltose, maltriose and other simple sugars [33].

Photographs of the lenses in normal and experimental groups are shown in **Fig. 4,** which revealed that the normal lens incubated with the artificial aqueous humor solution and glucose (5.5 mM) showed complete transparency. In the negative control, where the lens was incubated with glucose (55 mM), a complete opacification of the lens was noticed. Groups 3, in which the lens was incubated simultaneously with glucose (55 mM) and the Ethylacetate root extract of *Abutilon indicum* (500 µg/ml), showed a considerable reduction in the opacity of the lens similar to that of Group 4 treated with standard drug. The result indicates a positive effect of the selected plant extract on anticataract potential by exhibiting a reduction in the opacity of cataractous lenses. It is evident from **Table 4**, **Fig. 5** that there was a significant decrease in the level of total protein and an increase in the level of MDA in the cataractous lens (Group 2) compared to normal control (Group 1). The lens with the plant extract (Group 3) and the lens treated with the standard drug enalapril caused a significant increase in the total protein and a decrease in the level of MDA. A cataract is the most prevalent disorder leading to visual impairment. Pharmacological intervention to inhibit or to delay lens Opacification is yet at the experiment stage. Several factors are involved in the induction of this disease process, but the exact mechanism of cataract formation is still unclear. Studies are ongoing to explore the mechanism of cataractogenesis using various cataract models. Among various experimental models, the galactose model is commonly used, as it produces a greater increase in its reduced form, galactitol, than glucose, and galactitol does not further metabolize as does sorbitol, the reduced form of glucose [34]. Galactose model is reasonable to assume that the factors initiating the galactose cataract in young rats are very similar to those involved in the human galactose cataract [35]. The lens opacities in rats fed galactose, like those in human galactosemic subjects, slowly disappear when rats are placed on diets free of galactose. Three possible mechanisms that may be involved in cataract formation due to hyperglycemia or hypergalactosemia are the polyol pathway, oxidation, and non-enzymatic glycation [36].

Alkaloids, tannins, and Triterepenoid were found to be present in *Abutilon indicum* root; the anticataract activity associated with extract of this plant may be attributed to the presence of these constituents [37]. Sugar cataract formation is associated with diabetes and galactosemia has been linked to the aldose reductase catalyzed production of polyols, sorbitol, and galactitol from glucose and galactose respectively. Accumulation of high concentrations of polyols in the lens leads to excessive hydration, gain of sodium, and loss of potassium ions due to an increase in intracellular ionic strength [38]. Also, there is a loss of membrane permeability and leakage of free amino acids, glutathione, myoinositol and other small molecular weight substances. The resulting hyper osmotic stress associated oxidative insult is postulated to be the primary cause for the development of diabetic complications such as cataract [39]. Evidence has shown that there was a significant raise in polyols in galactosemic rats. In the present investigation polyol level was significantly decreased in ethylacetate extract of *Abutilon indicum* root treated rat lenses **Fig. 6.**

The anticataractogenic effect of ethylacetate extract of *Abutilon indicum* root was confirmed from the results of the study. In the present study, oral administration of ethylacetate extract of *Abutilon indicum* root showed significant protection against cataract formation in treated rats. The anticataract

potential of ethylacetate extract of *Abutilon indicum* root seems to be related to its antidiabetic property, as evident from Aplha amylase inhibition and antioxidant activity results.

**CONCLUSION:** In conclusion, ethylacetate extract of *Abutilon indicum* root showed anticataract activity against galactose cataracts in experimental animals along with good antioxidant and alpha-amylase inhibition. This preliminary study is encouraging, but further study is required to extrapolate the use of *Abutilon indicum* root in human beings for prophylaxis or treating human cataractogenesis.

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