

ASSESSMENT OF ANTIOXIDANT ACTIVITIES, PHENOL AND FLAVONOID CONTENTS OF DIFFERENT EXTRACTS OF LEAVES, BARK, AND ROOT FROM THE *ABUTILON INDICUM* (L.) SWEET

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ABSTRACT

Objective: This study antioxidant properties of petroleum ether, chloroform, ethyl acetate, and methanol extracts of *Abutilon indicum* (L).

Methods: The different extracts of *A. indicum* leaves, bark, and roots were antioxidant potential using 1,1-diphenyl-2-picryl-hydrazyl, 2, 2-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS⁺), hydrogen peroxide scavenging, superoxide anions scavenging, hydroxyl radical scavenging, ferric reducing antioxidant power, total antioxidant activity (phosphomolybdic acid) and compare to standard for L- ascorbic acid, butylated hydroxytoluene, and gallic acid.

Results: The highest antioxidant activities were recorded in ethyl acetate extracts of leaves of *A. indicum* followed by roots and bark. Among the plant parts, the ethyl acetate extract of leaves exhibited a significant effect in comparison with other solvent extracts of parts of plant. The ethyl acetate extracts showed the highest total phenol and flavonoid contents in leaves extracts of *A. indicum*. The total phenol (3.08±0.06) mg/ml and flavonoid (7.16±0.15) mg/ml were found to be higher in ethyl acetate extract of *A. indicum*.

Conclusion: The results of the study revealed that the ethyl acetate extract of *A. indicum* leaves can be used for the biological characterization and importance of the compounds identified and creates a platform to screen many bioactive compounds to treat many diseases.

Keywords: *Abutilon indicum*, Free radical scavenging activity, 1,1-diphenyl-2-picryl-hydrazyl, 2-azino-bis-3-ethyl benzthiazoline-6-sulfonic acid, Ferric reducing antioxidant power.

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INTRODUCTION

Free radicals are continuously produced by the body's normal use of oxygen [1]. Oxygen is an element indispensable to life. When cells use oxygen to generate energy, free radicals are produced by the mitochondria. These by-products are generally reactive oxygen species as well as reactive nitrogen species that result from the cellular redox process [2]. Free radicals can be either harmful or helpful to the body. When there is an imbalance in the formation and removal of free radicals then a condition called as oxidative stress is developed in the body. To counteract these free radicals, the body has protective antioxidant mechanisms with the abilities to lower incidence of various human morbidities and mortalities [3].

Antioxidants induce oxidative damage to biomolecules such as lipids, proteins, and DNA. This damage has been implicated in cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation, and other diseases [4-6]. Antioxidants are the substances that may protect the cells from the oxidative damage caused by free radicals. Natural products have been strong antioxidant activity and they have potential beneficial effects on human health. Many plant species and their active principles have been investigated in the search for natural antioxidants with pharmacological properties [7,8]. The studies of medicinal plants not end just with knowledge of their therapeutic use. Various aspects of medicinal plants have to be studied depth for their optimum utilization plant with possible antioxidant activity and to as certain be parameters associated with it.

Abutilon indicum (L.) Sweet belongs to the family Malvaceae and distribute in all parts of tropical and subtropical region of India. It is a

perennial shrub, softly tomentose and up to 3 m in height. Tamil name: Thuthi Sanskrit name: *Atibalaa*, Telugu name: *Duvvena Kayalu*. All parts of the plant have been recognized to have medicinal properties. The leaves are ovate, acuminate, toothed, rarely subtrilobate and 2.5 cm long. The flowers are yellow in color, peduncle jointed above the middle. The petioles are 3.8-7.5 cm long; stipules 9 mm long; pedicels often 2.5-5 mm long, axillary solitary, jointed very near the top; calyx 12.8 mm long, divided into middle, lobes ovate, apiculate and corolla 2.5 cm in diameter, yellow, opening in the evening. The fruits are capsule, densely pubescent, with conspicuous and horizontally spreading beaks. The stems are stout, branched, 1-2 m tall, pubescent. The seeds are 3-5 mm, reniform, tubercled or minutely stellate-hairy, black or dark brown 5-8.

The traditional method of medicine, the plant used as anthelmintic, anti-inflammatory and antioxidant are useful in urinary and uterine discharges, piles and lumbago [9], jaundice, ulcer, and leprosy. *A. indicum* leaves are used in the treatment of toothache, lumbago, piles, antifertility, and liver disorders [10]. Root and bark are used as aphrodisiac, antidiabetic and antioxidant activities [11], nervine tonic, and diuretic. The plant extracts and their products for antimicrobial and antioxidant activities have shown that a potential source of novel antibiotic prototypes of higher plants [12].

Hence, the aim of this research was made to study the antioxidant activities of different extracts of leaves, bark, and roots from the *A. indicum*.

METHODS**Collection of plant material**

The leaf, bark, and root of *A. indicum* (L.) Sweet was collected from Kadavachery village (Lat, 11.24 °N; Long, 79.44 °E), Cuddalore

District, Tamil Nadu, India during the month of January 2014. Herbarium was deposited in the Department of Botany, Annamalai University (Voucher specimen No; AUBOT #325). The different parts were washed with tap water, then surface sterilized with 10% sodium hypochloride solution to prevent contamination. The samples were rinsed with distilled water and allowed to shade dried under room temperature followed by oven drying at 50°C and then ground into powder using electric blender.

Preparation of extraction

About 100 g of powdered material of leaf, bark, and root samples were extracted in a Soxhlet apparatus for 8 hrs with different solvents system such as petroleum ether, chloroform, ethyl acetate, and methanol. The extracts were filtered, pooled, and the solvents were evaporated with the help of rotary evaporator (Heidolph, Germany) under reduced pressure at 40°C and the crude extracts were kept at 4°C in refrigerator for further analysis.

1, 1-diphenyl - 2 - picrylhydrazyl hydrate (DPPH) radical scavenging activity

The DPPH radical scavenging activities of the different extracts of plant parts were evaluated by the method [13]. Different extracts of various parts samples (0.1 ml) at various concentrations (125, 250, 500 and 1000 µg/ml) was mixed with 1 ml of 0.2 mM DPPH dissolved in methanol. The reaction mixture was incubated for 20 minutes at 28°C in the dark. The control contained all the reagents without the leaf sample and was used as blank. The DPPH radical scavenging activities were determined by measuring the absorbance at 517 nm using a spectrophotometer (Hitachi U-20). Vitamin C was used as positive control. The antioxidant activities of plant extracts were expressed as inhibitory concentration (IC₅₀), which was defined as the concentration (µg/ml) of extracts required to inhibit the formation of DPPH radicals by 50%. The DPPH radical concentration was calculated using the following equation.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

ABTS^{•+} scavenging effects (2, 2-Azino-bis-3-ethyl benzthiazoline-6-sulfonic acid)

The antioxidant effect of the different crude extracts of *A. indicum* parts was evaluated by the method [14]. ABTS^{•+} radical cations (ABTS^{•+}) were produced by reacting ABTS^{•+} solution 7 mM with 2.45 mM potassium persulfate. The mixture was incubated at room temperature in the dark for 12-16 hrs to yield a dark-colored solution containing ABTS^{•+} radicals and diluted. The different concentrations of (125, 250, 500 and 1000 µg/ml) extracts were added to 1 ml of ABTS^{•+} solution. The absorbance was read at 734 nm after 6 minutes in a spectrophotometer (Hitachi U-20). Butylated hydroxyl toluene (BHT) was used as the standard. Appropriate solvents blanks were run in each assay. All determinations were carried out in triplicate, and the percent of inhibition was calculated using the formula.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydrogen peroxide scavenging effects

The ability of the different extracts of different parts samples were evaluated by the method [15]. A solution of H₂O₂ (40 mM) was prepared in phosphate buffer. Different crude extracts at the various concentrations of (125, 250, 500 and 1000 µg/ml) were added to H₂O₂ solution (0.6 ml) and the total volume was made up to 3 ml. The absorbance of the reaction mixture was recorded at 230 nm in a Spectrophotometer (Hitachi U-20). A blank solution containing phosphate buffer, Vitamin C was used as positive control. The extent of H₂O₂ scavenging of the different extracts were calculated using the formula.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Superoxide anions radical scavenging activity

The superoxide anions scavenging ability of the different crude extracts were assessed by the method [16]. Superoxide anions were generated in parts samples that contained in 3.0 ml, 0.02 ml different crude extracts at the concentrations of (125, 250, 500 and 1000 µg/ml) 0.2 ml of ethylenediaminetetraacetic acid (EDTA), 0.1 ml of NBT, 0.05 ml of riboflavin and 2.64 ml of phosphate buffer. The control tubes were also set up where dimethyl sulfoxide was added instead of the plant extracts. All the tubes were vortexed and the initial optical density was measured at 560 nm in a spectrophotometer (Hitachi U-20). Vitamin C was used as positive control. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activities.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging from Fenton reaction was quantified using 2'-deoxyribose oxidative degradation as described [17]. The reaction mixture contained 0.1 ml of deoxyribose, 0.1 ml of FeCl₃, 0.1 ml of EDTA, 0.1 ml of H₂O₂, 0.1 ml of ascorbate, 0.1 ml of KH₂PO₄-KOH buffer (125, 250, 500 and 1000 µg/ml) of plant extracts of various concentrations in a final volume of 1.0 ml. The mixture was incubated at 37°C for 1 hr. At the end of the incubation period, 1 ml of thiobarbituric acid (TBA) was added and heated at 95°C for 20 minutes to develop the color. After cooling, the TBA formation was measured spectrophotometrically (Hitachi U-20) at 532 nm against an appropriate blank. The hydroxyl radical scavenging activities were determined by comparing the absorbance of the control with samples. The percent TBA production for positive control Vitamin C was fixed at 100% and the relative percent TBA was calculated for the extracts.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Total antioxidant activity (phosphomolybdic acid method)

The antioxidant activities of the samples parts were evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [18]. Aliquots of 0.4 mL of sample solution were combined in a vial with 4 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95°C for 90 minutes. After the sample had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activities were expressed relative to that of Vitamin C.

$$\text{Percentage of inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Ferric reducing antioxidant power

The ferric reducing antioxidant potential of various crude extracts of sample parts as per the method [19]. The samples were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric cyanide. After the mixture was incubated at 50°C for 20 minutes, 2.5 ml of 10% trichloroacetic acid, 2.5 ml distilled water, and 0.5 ml of 0.1% ferric chloride was added and then the absorbance was measured at 700 nm against a blank. The blank consist of all the reagents without the test sample. The reducing power of gallic acid was also determined

for a comparison. High absorbance of the reaction mixture indicates strong ferric reducing antioxidant power.

Total phenol content

Total phenolic content was carried out following the Folin-Ciocalteu method described [20]. 1 ml of crude sample extracts solution containing (1 mg/ml) was added volumetric flask. 1 ml of Folin-Ciocalteu reagent and allowed to stand at 22°C for 5 minutes; 7.5% of 0.75 ml of sodium bicarbonate solution was added and mixed thoroughly. The samples were measured spectrophotometrically (Hitachi U-20) at 765 nm using spectrometer after 90 minutes at 22°C. The amount of total phenolic was determined as gallic acid and equivalent and expressed as mg gallic acid equivalent (GAE/g).

Total flavonoid content

The flavonoids content was determined by aluminum trichloride method using catechin as a reference compound [21]. This method based on the formation of a complex flavonoid-aluminum having the absorptive spectrophotometrically (Hitachi U-20) maximum at 415 nm, after remained react at room temperature for 30 minutes. Briefly, 0.5 mL of each extracts (1:10 g/mL) in methanol was separately mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The amount of total flavonoid was determined as mg QE/g.

Statistical analysis

The results are expressed as the mean \pm standard deviation. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between different extracts for *in vitro* antioxidant activity assays. Comparison of means for *in vivo* antioxidant activity assessment was carried out using one-way analysis of variance and Duncan test. $p < 0.05$ was considered statistically significant.

RESULTS

The petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest DPPH activity. The IC_{50} values of ethyl acetate extracts of *A. indicum* leaf, bark, root, and Vitamin C (standard) values were ranged from 347.12, 421.34, 438.10, and 204.14 $\mu\text{g/ml}$. The IC_{50} values of methanol extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 410.14, 498.36, 486.95, and 198.48 $\mu\text{g/ml}$. The IC_{50} values of chloroform extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged between 426.18, 736.80, 735.57, and 192.48 $\mu\text{g/ml}$. The IC_{50} values of petroleum ether extract of *A. indicum* leaf, bark, and root, and Vitamin C values were ranged from 843.32, 954.84, 1047.12, and 209.36 $\mu\text{g/ml}$ is shown in Fig. 1. In this study, different leaf extracts of *A. indicum* exhibited the highest ABTS^{•+} activity. The IC_{50} values of ethyl acetate extract of *A. indicum* leaf, bark, root, and standard BHT values were ranged between 295.72, 454.16, 368.42, and 209.36 $\mu\text{g/ml}$, respectively (Fig. 2). The different extracts of leaf from the *A. indicum* exhibited the highest superoxide anion activity. The IC_{50} values of ethyl acetate extracts of *A. indicum* leaf, bark, root, and Vitamin C (standard) values were ranged from 958.09, 1225.46, 1086.23, and 642.59 $\mu\text{g/ml}$. The IC_{50} values of methanol extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 1052.28, 1368.47, 1124.78, and 656.48 $\mu\text{g/ml}$. The IC_{50} values of chloroform extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 1138.49, 1534.52, 1554.13, and 656.12 $\mu\text{g/ml}$. The IC_{50} values of petroleum ether extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 1152.56, 1885.26, 1662.38, and 684.78 $\mu\text{g/ml}$ is shown Fig. 3.

The petroleum ether, chloroform, ethyl acetate, and ethyl acetate leaf extracts of *A. indicum* exhibited the highest ferric reducing antioxidant power. The IC_{50} values of ethyl acetate extracts of *A. indicum* leaf, bark, root, and gallic acid (standard) values were ranged from 636.35,

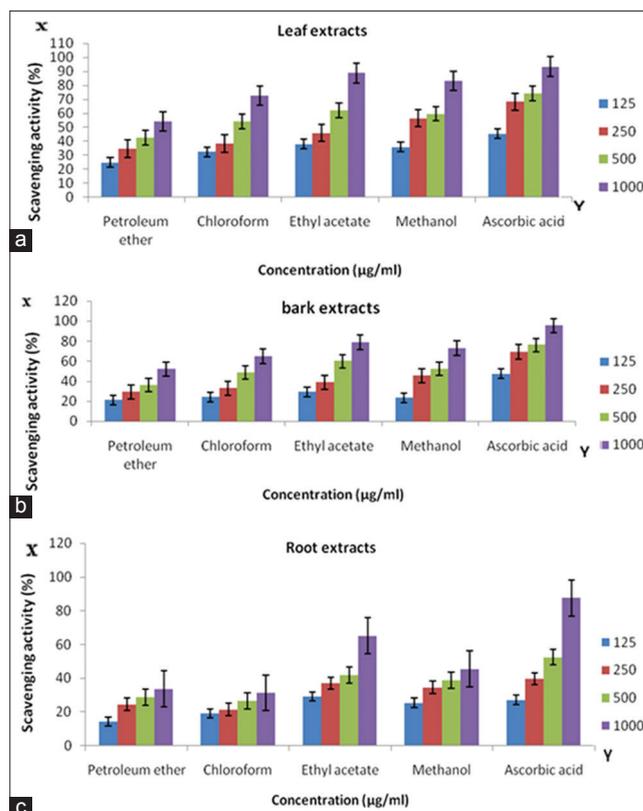


Fig. 1: (a-c) 1,1-diphenyl-2-picrylhydrazyl hydrate radical scavenging activity of various extracts of *Abutilon indicum*

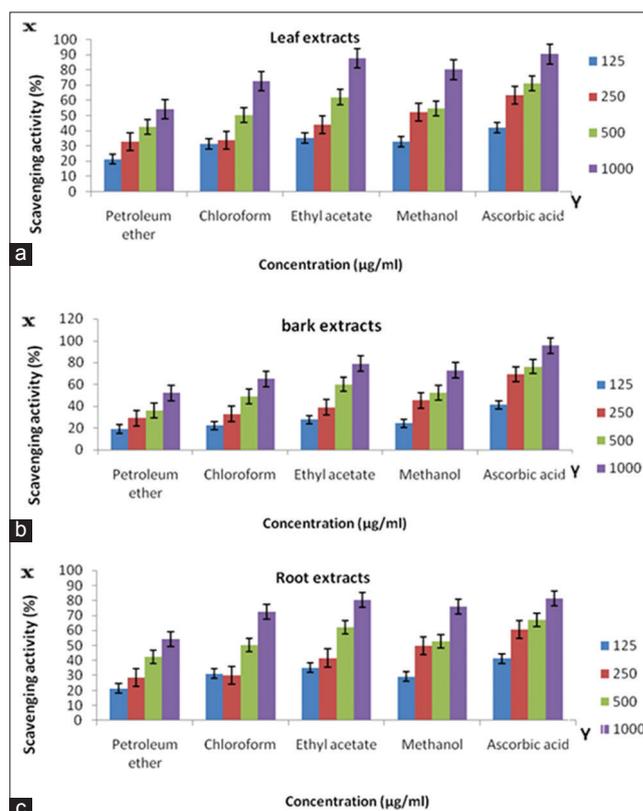


Fig. 2: (a-c) 2,2-Azino-bis-3-ethyl benzthiazoline-6-sulfonic acid (ABTS^{•+}) of various extracts of *Abutilon indicum*

934.67, and 494.15 µg/ml is shown in Fig. 4. The different extracts of *A. indicum* exhibited the highest hydroxyl radical activity. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) values were ranged from 1032.15, 1153.38, 1120.45, and

984.17, 934.67, and 494.15 µg/ml is shown in Fig. 4. The different extracts of *A. indicum* exhibited the highest hydroxyl radical activity. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) values were ranged from 1032.15, 1153.38, 1120.45, and 945.78 µg/ml. The IC₅₀ values of methanol extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 1094.76, 1184.31, 1106.38, and 918.42 µg/ml. The IC₅₀ values of chloroform extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 1234.47, 1238.53, 1212.47, and 898.42 µg/ml. The IC₅₀ values of petroleum ether extract of *A. indicum* leaf, bark, root, and Vitamin C values were ranged from 2034.46, 2158.47, 2132.68, and 930.12 µg/ml is shown in Fig 5. The petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest total antioxidant. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) were 848.12, 986.20, 1037.58, and 657.42 µg/ml, respectively (Fig. 6). The different extracts of *A. indicum* exhibited the highest H₂O₂ activity. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) values were found to be 1034.34, 1068.76, 1164.35, and 942.39 µg/ml. The IC₅₀ values of methanol extract of *A. indicum* leaf, bark, root, and Vitamin C values were found to be 1124.78, 1156.47, 1258.47, and 962.27 µg/ml. The IC₅₀ values of chloroform extract of *A. indicum* leaf, bark, root, and Vitamin C values were found to be 1532.36, 1248.86, 1136.83, and 934.12 µg/ml. The IC₅₀ values of petroleum ether extract of *A. indicum* leaf, bark, root, and Vitamin C values were found to be 1051.15, 1074.60, 1168.97, and 429.84 µg/ml is shown in Fig. 7.

Total phenol and flavonoid contents were extracted by organic solvents with different polarities of petroleum ether, chloroform, ethyl acetate, and methanol. The results are presented in Tables 1 and 2 showed the differences in the total phenolic and flavonoid content in different parts of *A. indicum*. The total phenols (3.08±0.06) µg/ml GAE/g and flavonoids (7.16±0.15) µg/ml quercetin equivalent (QE/g) were found to be higher in ethyl acetate extract of leaves of *A. indicum*.

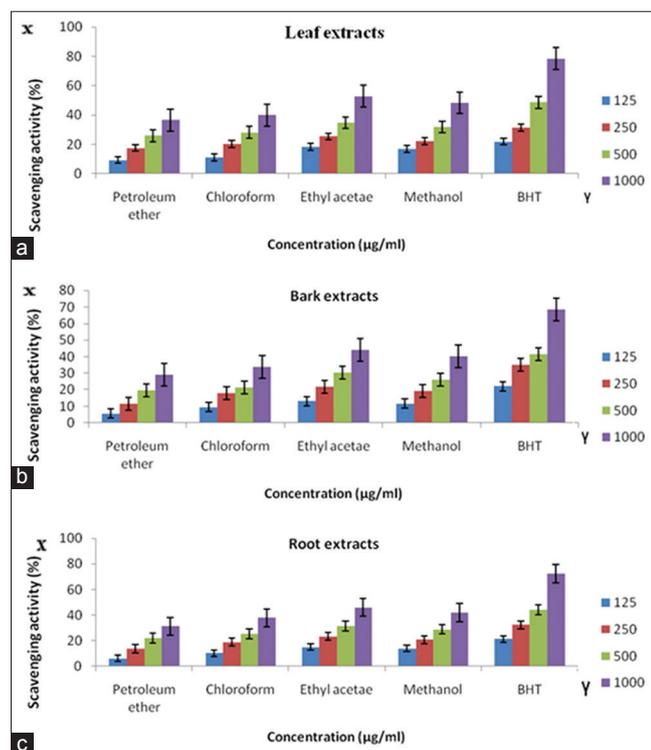


Fig. 3: (a-c) Superoxide anion scavenging activity of different extracts of *Abutilon indicum*

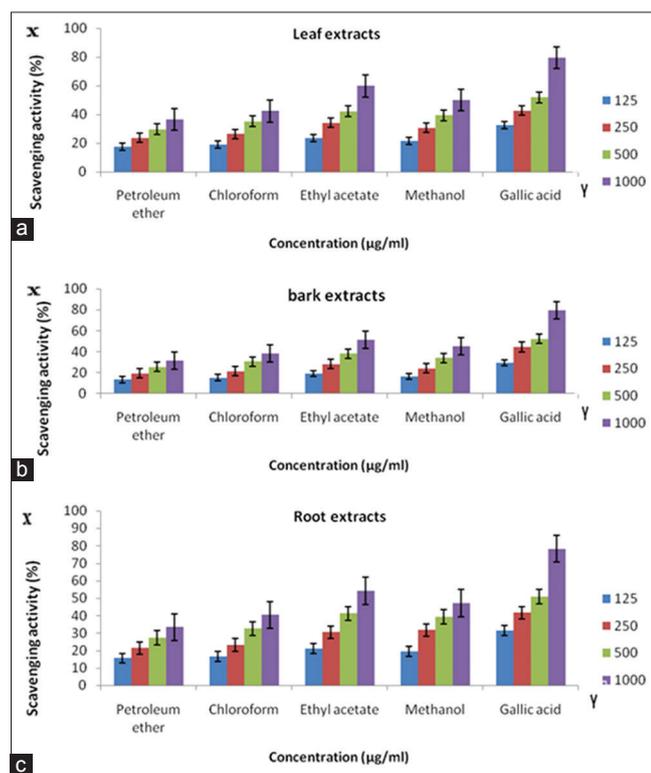


Fig. 4: (a-c) Ferric reducing antioxidant power of various extracts of *Abutilon indicum*

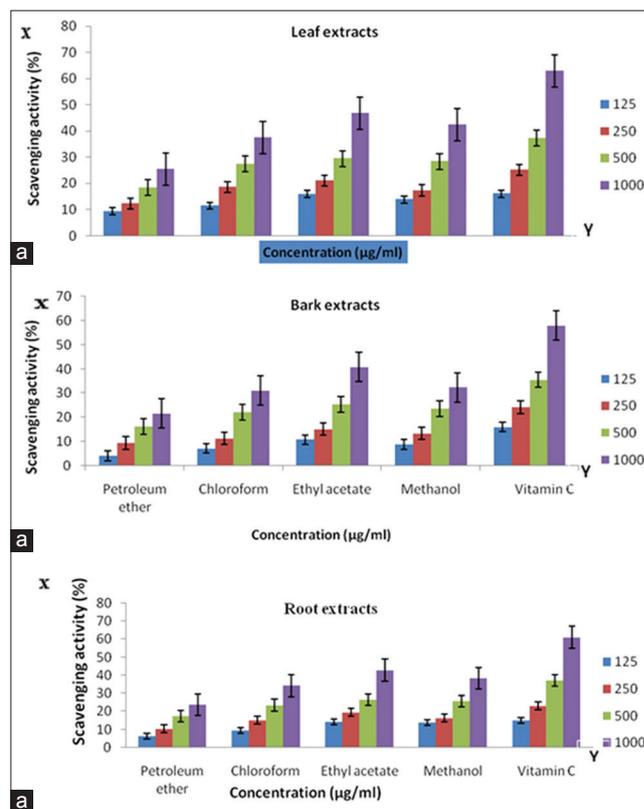


Fig. 5: (a-c) Hydroxyl radical scavenging activity of different extracts of *Abutilon indicum*

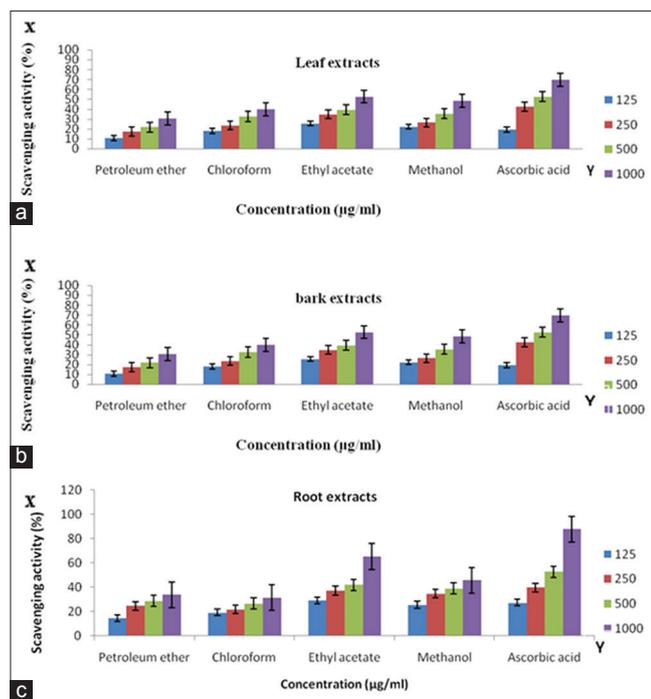


Fig. 6: (a-c) Total antioxidant activity of various extracts of *Abutilon indicum*

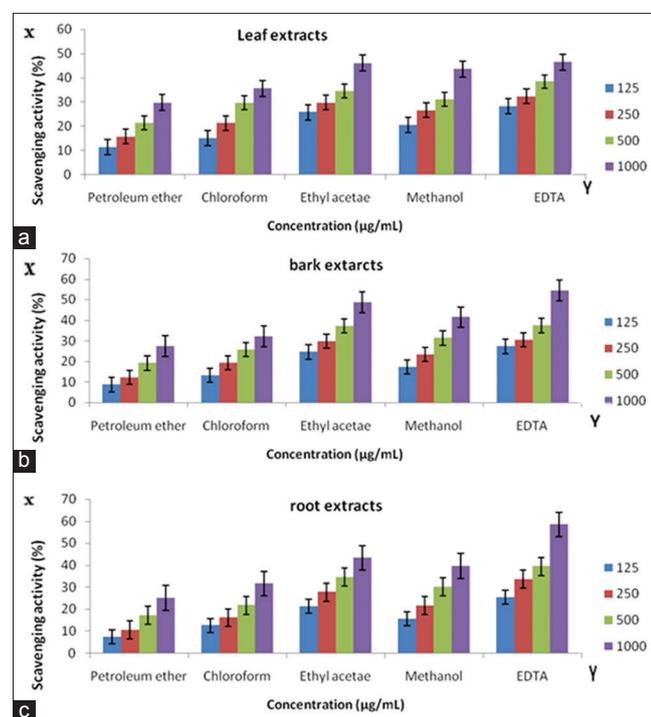


Fig. 7: (a-c) Hydrogen peroxide scavenging activity of various extracts of *Abutilon indicum*

DISCUSSION

In this study, petroleum ether, chloroform, ethyl acetate, and methanol extracts of *A. indicum* leaves, stem and root. Among the ethyl acetate extract was found to be the most effective solvent in extraction of antioxidants from the leaves. The ethyl acetate is a widely used and effective solvent for extraction of antioxidant. Ethyl acetate extracts proved to be the most efficient solvent for extraction of antioxidants from *Pleurotus florida* as the related extract contained the highest amount

Table 1: The total phenol content of different extracts of *A. indicum*

Total phenol GAE µg/ml ^c			
Solvents	Leaf	Bark	Root
Petroleum ether	0.64±0.12 ^a	0.34±0.12 ^a	0.40±0.05 ^a
Chloroform	0.75±0.08 ^a	0.45±0.31 ^a	12.05±0.04 ^a
Ethyl acetate	3.08±0.06 ^b	1.64±0.02 ^a	14.71±0.15 ^{ab}
Methanol	2.05±0.52 ^b	1.30±0.28 ^{ab}	15.03±0.96 ^b
Gallic acid	6.82±0.05 ^c	3.32±0.78 ^b	23.3±0.41 ^c

^aAll the values are mean±SD, SD: Standard deviation, ^{ab,c}different letters in the same column indicates significant difference of superscript by DMRT test at p<0.05, ^ctotal phenolic contents were expressed as gallic acid equivalents (GAE/g) samples, GAE: Gallic acid equivalent

Table 2: The total flavonoid content of different extracts of *A. indicum*

Total flavonoid QE µg/ml ^c			
Solvents	Leaf	Bark	Root
Petroleum ether	3.50±0.10 ^a	1.05±0.18 ^a	2.80±0.50 ^a
Chloroform	3.90±0.18 ^a	0.37±0.25 ^a	2.49±0.04 ^a
Ethyl acetate	7.16±0.15 ^{ab}	5.41±0.12 ^a	6.26±0.09 ^{ab}
Methanol	6.89±0.04 ^b	4.19±0.21 ^a	5.43±0.21 ^a
Quercetin	11.12±0.09 ^c	7.45±0.25 ^c	9.26±0.03 ^c

^aAll the values are mean±SD, SD: Standard deviation, ^{ab,c}different letters in the same column indicates significant difference of superscript by DMRT test at p<0.05, ^ctotal flavonoid contents were expressed as quercetin equivalents (QE/g) samples

of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays [22]. This study petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest DPPH activity. Similar results were observed Nayak *et al.* [23] coincide the results DPPH assay this process changes the characteristic purple color of the DPPH solution to yellowish. The colorimetric method involves decrease in absorbance by increasing activity of the plant extract to neutralizing free radicals generated by the DPPH. *A. indicum* and *Paederia foetida* both shows decrease in absorbance but *A. indicum* has higher power of inhibition compared to *P. foetida* as former showed the IC₅₀ value 52 µg/ml and later shows IC₅₀ value 98 µg/mL. Kumar *et al.* [24] reported that *in vitro* antioxidant activity of methanolic extract of aerial parts of *Salvia splendens* had shown very significant DPPH radical scavenging, hydrogen peroxide scavenging, and superoxide anion scavenging activity compared to standard.

In this study, petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest ABTS^{•+} activity. Jamuna and Ravishankar [25] reported that the chloroform and ethyl acetate extracts of root part of *Hypochoeris radicata* exhibited higher ABTS^{•+} radical scavenging activity. On the other hand, in our results, the ABTS^{•+} activity of leaf, bark, and root extracts of *A. indicum* was shown in the order of ethyl acetate > methanol > chloroform > petroleum ether. The results are in agreement with that of the previous reports of Whistler and BeMiller [26]. Chen *et al.* [27] studied the antioxidant activities of five medicinal plants, viz., *Ampelopsis sinica*, *Ampelopsis humilifloria*, *Potentilla freyniana*, *Selaginella labordei*, and *Chrysanthemum multiflorum*. Extracts from all five of the plants inhibited xanthine oxidase and lipoxygenase activities and were scavengers of the ABTS^{•+} radical cation using the trolox equivalent antioxidant capacity assay. Surveswaran *et al.* [28] reported that 133 plants belongs to the 64 families were collected and their extracts were tested for their antioxidant capacity. DPPH radical scavenging, ABTS^{•+} radical scavenging and ferric reducing antioxidant power activity. The total phenol contents were measured in each plant tissue, as well as individual phenolics to identify the compounds potentially responsible for the strongest antioxidant activities.

The petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest superoxide anion activity. Super oxide is a highly reactive molecule that reacts with various substances produced through the metabolic process, superoxide dismutase enzyme present in aerobic and anaerobic organisms catalyses the breakdown of superoxide radical [29]. The superoxide scavenging ability of plant extract might primarily be due to the presence of flavonoids [30]. Superoxide anion is one of the most representative free radicals. In cellular oxidation reactions, superoxide radicals have their initial effects magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents, e.g., hydroxyl radical [31]. Antioxidant activity of methanolic extract of *A. indicum* leaves was investigated for its free radical scavenging activity by determining the nitric oxide and super oxide radical scavenging activity.

In this study, petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest ferric reducing antioxidant power. The ferric reducing antioxidant power (FRAP) assay showed reducing powers of the fraction in order of butanol > ethyl acetate > chloroform > *n*-hexane or *Acaulon muticum*, respectively. The reaction kinetics with this free radical indicated the presence of both slow reacting and fast reacting antioxidant components in the extracts of both plants. *Abutilon* species are potential sources of natural antioxidants [32]. Ahmed and Urooj [33] suggested that the antioxidant activity of leaf extracts of *A. indicum* was evaluated to explore new bioactive compounds with least associated side effects. The methanol extracts were prepared and screened for *in vitro* using FRAP. The reducing power of methanolic leaf extract was markedly increased by increasing concentration. The results indicated a strong antioxidant activity.

The petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest total antioxidant. Ashafa et al. [34] have reported the total antioxidant activity of the acetone extracts of *Felicia muricata*. It was found to be a good total antioxidant activity due to the presence of gallic acid. The antioxidant activity of methanolic extracts from the leaves and stem of *Mollugo nudicaulis* were by quantifying phenolic, flavonoids contents and by antioxidant assays such as DPPH and ferric reducing power assays. The results indicate that presence of significant quantities of total phenolics, flavonoids, and antioxidant activity [35].

This study petroleum ether, chloroform, ethyl acetate, and methanol leaf extracts of *A. indicum* exhibited the highest hydroxyl radical activity. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) were 1032.15, 1153.38, 1120.45, and 945.78 µg/ml, respectively. Hydroxyl radical is highly reactive oxygen, centered radical, formed from the reaction of various hydroperoxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acid membranes and most biological molecules it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxide reaction of lipids [36].

The different extracts of *A. indicum* exhibit the highest H₂O₂ activity. The IC₅₀ values of ethyl acetate extract of *A. indicum* leaf, bark, root, and Vitamin C (standard) were found to be 1034.34, 1068.76, 1164.35, and 942.39 µg/ml, respectively. Dhruvi [37] reported that the antioxidant activity of the methanolic and aqueous extracts of *Martynia annua* leaves was evaluated by several *in vitro* systems of assay namely, reducing power assay, DPPH radical scavenging activity, nitric oxide scavenging activity, H₂O₂ radical scavenging activity, superoxide radical scavenging, hydroxyl radical-scavenging activity, and total antioxidant capacity. The total phenolic content was measured by Folin-Ciocalteu reagent. The antioxidant property depends on concentration and increased with increasing amount of the extract. The free radical scavenging and antioxidant activity may be attributed to the presence of phenolic and flavonoid compounds present in the extract. The results showed that the ethyl acetate extract exhibited higher antioxidant activity than the methanol, chloroform, and hexane extracts.

The total phenols (3.08±0.06) µg/ml GAE/g and flavonoids (7.16±0.15) µg/ml quercetin equivalent (QE/g) were found to be higher in ethyl acetate extract of leaves of *A. indicum*. The phenolic compounds are known as powerful chain breaking antioxidant. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl group and may contribute directly to antioxidative action [38]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [39]. Similar results were observed total phenolic content of 154.81 mg GAE/g in the water extracts and 169.06 mg GAE/g in methanol extract leaves of *Teucrium montanum* L. Var. *montanum*, F. *supinum* of leaves was reported [40]. The leaf ethyl acetate extract of *T. montanum* had flavonoid content of 58.48 mg RU/g. Flavonoids are potent antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity [41]. The leaf extracts exhibit a much higher total phenol and flavonoid contents when compared to the bark and root. These results probably explain the high antioxidant activities found in aerial parts of leaves and stem and are in agreement with previous findings reported in other medicinal plants [42].

CONCLUSION

The natural products proved less side effects and cure diseases effectively from ancient period. In this study, the crude extracts of *A. indicum* leaves, bark, and root. In that way, medicinal plant, *A. indicum* leaves can be used as elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases.

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