



## Screening of Phytochemical, *in vitro* Antioxidant and Antibacterial Activities of Stem and Root Extracts of *Abutilon indicum* (L.) SW

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### ABSTRACT

The present study was conducted to evaluate the qualitative and quantitative phytochemical analysis, FT-IR, *in vitro* antioxidant and antibacterial activity of *Abutilon indicum* stem and root. Qualitative phytochemical analysis of methanol extracts of *A.indicum* stem and root revealed the presence of anthraquinone, catechin, coumarin, quinine, sugar, carbohydrate and xanthoprotein whereas the ethanol extracts of *A.indicum* stem and root possessed the presence of coumarin, flavonoid, steroid, tannin, carbohydrate and xanthoprotein. Among the solvent tested, ethanol extract exhibited highest total phenolic content whereas methanol extracts showed the highest total flavonoid contents. FT-IR peaks obtained confirms hydroxyl, alkyl, tertiary amine salt, amino acids, aromatic ether, chloro compounds. Methanol extracts of stem and root of *A.indicum* exhibited highest DPPH radical scavenging activity. Ethanol extract of *A.indicum* stem showed highest hydroxyl radical scavenging activity whereas methanol extract of root showed highest hydroxyl radical scavenging activity. Similarly petroleum ether extract of stem possessed highest superoxide radical scavenging activity whereas methanol extract of root showed highest superoxide radical scavenging activity. Like the antioxidant activity, reducing power of the extract increase with increase in concentration. In the present study, the methanol extracts of stem and root exhibited highest reducing ability. In the present study, the ethyl acetate and methanol extracts of *A.indicum* stem and root showed the highest inhibitory activity against *K.pneumoniae*, *P.vulgaris* and *E.coli*, *K.pneumoniae* respectively.

**Keywords:** Phytochemical, FT-IR, antioxidant, antibacterial, flavonoid.

### INTRODUCTION

Reactive Oxygen Species (ROS) are constantly formed in the human body by normal metabolic action and there are exert oxidative damaging effects by reacting with nearly every molecule found in living cells including nucleic acids, proteins, lipids or DNA and may involve in several chronic and degenerative diseases including gastritis, reperfusion injury of many tissues, atherosclerosis, ischemic heart diseases, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others<sup>1,2</sup>, if excess ROS and free radicals are not eliminated by endogenous antioxidant system. Although the body possesses such defence mechanisms, as enzymes and antioxidants nutrients, which arrest the damaging properties of ROS.<sup>3,4</sup>

Currently, the possible toxicity of synthetic antioxidants has been criticized. It is generally assumed that frequent consumption of plant derived phytochemicals from vegetables, fruit, tea and herbs may contribute to shift the balance toward an adequate antioxidant status.<sup>5</sup> Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.<sup>6</sup>

Plants are the good sources for the discovery of pharmaceutical compounds and medicines which are used to cure ailments of human beings with no side effects compared with synthetic drugs. They have many secondary metabolites which confer specific characteristics and properties to plants.<sup>7</sup> Plants and plant extracts have formed important position in modern medicine, due to their chemical and medicinal contents

found in the natural form. The secondary metabolites represent a large reservoir of structural moieties which work together exhibiting a wide range of biological activities. Phytochemicals especially polyphenols constitute a major group of compounds that act as primary natural antioxidants<sup>8</sup> and thus an interest has been increased considerably among scientists, drugs and food manufactures.<sup>9</sup> More than 4000 phenolics compounds (flavonoids, monophenols and polyphenols) are found in vascular plants. Phenolic compounds such as quercetin, rutin, naringin, catechin, caffeic acid, gallic acid and chlorogenic acid are very important plant constituents.<sup>10</sup> Recent research suggested that diets rich in polyphenolic compounds and flavonoids are associated with longer life expectancy<sup>11</sup> and found effective in many health-related properties, such as anticancer, antiviral, antiinflammatory activities, effects on capillary fragility and an ability to inhibit human platelet aggregation.<sup>12</sup> So, there is a growing importance in medicinal plants and traditional health systems providing health care for a wider population across the globe, especially, in the developing countries.

*Abutilon indicum* (Indian Abutilon, Indian Mallow) is a small shrub in the Malvaceae family, native to tropic and subtropical regions and sometimes cultivated as an ornamental. This plant is often used as a medicinal plant and is considered invasive on certain tropical islands. The plant is considered as astringent, antibacterial, anthelmintic, carminative and diuretic. It is used locally for colds, high fever, mumps, tuberculosis, bronchitis, diabetes, arbuticle, hemorrhoids, hernia, diarrhea and



various types of worm infections. In traditional medicine, *A.indicum* leaves are used as a demulcent, aphrodisiac, laxative, diuretic, pulmonary and sedative.

The bark is astringent and diuretic; seeds are laxative, expectorant and demulcent; whole plant is laxative and tonic, anti-inflammatory and anthelmintic; root is diuretic and leprosy.<sup>13</sup> The purpose of present study was to evaluate the phytochemical, *in vitro* antioxidant and antibacterial activity of five different solvent extracts of stem and root of *Abutilon indicum*.

## MATERIALS AND METHODS

### Collection of Plant Samples

Stem and root of *Abutilon indicum* (L.) SW were collected from Antonyar Puram, Thoothukudi, Tamil Nadu. With the help of local flora, voucher specimen were identified and preserved in the ethnopharmacology unit, Research department of Botany, V.O.Chidambaram College, Thoothukudi for further research.

### Preparation of Extracts for Phytochemical Analysis

Freshly collected stem and leaf samples of *Abutilon indicum* were dried in shade, and then coarsely powdered separately in a Wiley mill. The coarse powder (100g) were extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered through Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures.<sup>14-16</sup>

All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for estimation of total phenolic, flavonoid content and also used for *in vitro* antioxidant and antibacterial activity.

### FT-IR Analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a ThermoScientific Nicot iS5 iD1 transmission, between 4000 – 400 cm<sup>-1</sup>.<sup>17</sup>

### Estimation of Total Phenolic Content

Total phenolic contents were estimated using Folin-Ciocalteu reagent based assay as previously described<sup>18</sup> with little modification. To 1 mL of each extract (100 µg/mL) in methanol, 5 mL of Folin-Ciocalteu reagent (diluted ten-fold) and 4 mL (75 g/L) of Na<sub>2</sub>CO<sub>3</sub> were added. The mixture was allowed to stand at 20°C for 30 min and the absorbance of the developed colour was recorded at 765 nm using UV-VIS spectrophotometer. 1 mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve.

The absorbance of solution was compared with gallic acid

calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

### Estimation of Flavonoids

The flavonoids content was determined according to Eom.<sup>19</sup> An aliquot of 0.5ml of sample (1 mg/mL) was mixed with 0.1 mL of 10% aluminium chloride and 0.1 mL of potassium acetate (1 M). In this mixture, 4.3 ml of 80% methanol was added to make 5 mL volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 415 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

### DPPH Radical Scavenging Activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H.<sup>20</sup>

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method.<sup>20</sup> Briefly, an 0.1 mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 mL of the solution of all extracts at different concentration (50,100,200,400 & 800 µg/mL). The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability to scavenging the DPPH radical was calculated by using the following formula.

$$\% \text{ inhibition} = \{(A_0 - A_1)/A_0\} * 100$$

Where, A<sub>0</sub> is the absorbance of the control reaction, and A<sub>1</sub> is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged.

### Hydroxyl Radical Scavenging Activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell.<sup>21</sup> Stock solutions of EDTA (1 mM), FeCl<sub>3</sub> (10 mM), Ascorbic Acid (1 mM), H<sub>2</sub>O<sub>2</sub> (10 mM) and Deoxyribose (10 mM) were prepared in distilled deionized water.

The assay was performed by adding 0.1 mL EDTA, 0.01 mL of FeCl<sub>3</sub>, 0.1 mL H<sub>2</sub>O<sub>2</sub>, 0.36 mL of deoxyribose, 1.0 mL of the extract of different concentration (50,100,200,400 & 800 µg/mL) dissolved in distilled water, 0.33 mL of phosphate buffer (50 mM, pH 7.9), 0.1 mL of ascorbic acid in sequence. The mixture was then incubated at 37°C for 1 hour. 1.0 mL portion of the incubated mixture was



mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025 M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532 nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

#### Superoxide Radical Scavenging Activity

The superoxide anion scavenging activity was measured as described by Srinivasan.<sup>22</sup> The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3 mM), 0.5 mL NADH (0.936 mM) solution, 1.0 mL extract of different concentration (50,100,200,400 & 800 µg/mL), and 0.5 mL Tris – HCl buffer (16mM, pH 8.0).

The reaction was started by adding 0.5 mL PMS solution (0.12 mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

#### Antioxidant Activity by Radical Cation (ABTS +)

ABTS assay was based on the slightly modified method of Huang.<sup>23</sup> ABTS radical cation (ABTS+) was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use.

The ABTS + Solution were diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. After addition of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC).

The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

#### Reducing Power

The reducing power of the extract was determined by the method of Kumar and Hemalatha.<sup>24</sup> 1.0 mL of solution containing 50, 100, 200, 400 & 800 µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%):

The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm.

The experiment was performed thrice and results were averaged.

#### Microorganisms

Bacterial strains of *Escherichia coli* (-), *Salmonella paratyphi* (-), *Proteus vulgaris* (-) and *Klebsiella*

*pneumoniae* (-) bacterial strains were obtained from Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India. The bacteria were incubated on a nutrient agar-slant (Stationary cultures) for 48h at 37°C, followed by inoculation in Muller Hinton Agar (MHA) medium.

#### Antibacterial Assay

Antimicrobial study was carried out by disc diffusion method<sup>25</sup> against the pathogens. A loopful of bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with gram negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic streptomycin (30 mcg/disc) was used as reference or positive control. Plates were incubated at 37°C for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

#### Statistical Analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

## RESULTS

#### Qualitative and Quantitative Phytochemical Analysis

Qualitative and quantitative phytochemical analyses are shown in table 1. Methanol extract of *A.indicum* stem possessed anthraquinone, catechin, coumarin, quinine, steroid, terpenoid, sugar, carbohydrate and xanthoprotein. Similarly ethanol extract of *A.indicum* stem exhibited anthraquinone, coumarin, flavonoid, phenol, quinine, steroid, tannin, carbohydrate and xanthoprotein. The phytochemical screening of methanol extract of *A.indicum* root revealed the presence of alkaloid, anthraquinone, catechin, coumarin, phenol, quinine, sugar, carbohydrate and xanthoprotein whereas ethanol extract of *A.indicum* root possessed that catechin, coumarin, flavonoid, steroid, tannin, carbohydrate and xanthoprotein. Total phenol content of *A.indicum* stem and root was found to be higher in ethanol extracts and total flavonoid content of *A.indicum* stem and root was found to be higher in methanol extracts (Table 2).



**Table 1:** Preliminary Phytochemical Screening of Stem of *Abutilon indicum*

Test	Petroleum Ether		Benzene		Ethyl Acetate		Methanol		Ethanol	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root	Stem	Root
Alkaloid	-	-	-	-	+	-	-	+	-	-
Anthraquinone	-	-	-	-	+	-	+	+	+	-
Catechin	-	+	-	+	+	-	+	+	-	+
Coumarin	-	-	+	-	-	-	+	+	+	+
Flavonoid	+	-	-	+	+	-	-	-	+	+
Phenol	+	-	-	-	+	-	-	+	+	-
Quinine	+	-	-	-	-	-	+	+	+	-
Saponin	+	-	+	-	+	-	-	-	-	-
Steroid	-	-	-	-	-	-	+	-	+	+
Tannin	+	+	+	+	+	+	-	-	+	+
Terpenoid	-	-	-	-	-	-	+	-	-	-
Sugar	-	+	-	+	-	+	+	+	-	-
Carbohydrate	+	+	+	+	+	+	+	+	+	+
protein	+	+	+	+	+	+	+	-	-	+
Glycoside	-	-	+	+	-	+	-	-	-	-
Xanthoprotein	-	-	-	-	+	+	+	+	+	+
Fixed oil	+	+	+		+	+	-	-	-	-

+ Present - absent

**Table 2:** Quantitative Analysis of Total Phenol and Flavonoid in Stem and Root of *A.indicum*

Plant Parts	Solvents	Total Phenol (mg/g)	Total flavonoid (mg/g)
Stem	Methanol	1.17	1.55
	Ethanol	1.87	1.68
Root	Methanol	1.24	1.26
	Ethanol	1.94	1.78

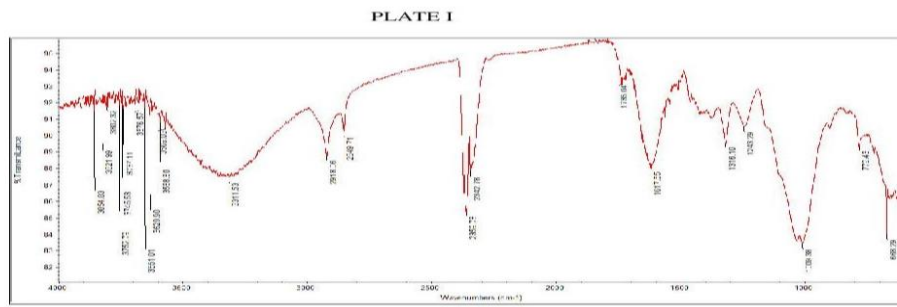
**FT-IR**

The FT-IR spectrum of *A.indicum* stem and root were given in plate I. The data on the peak values and the probable functional groups present in the powdered sample of *A.indicum* stem and root were presented in Table 3.

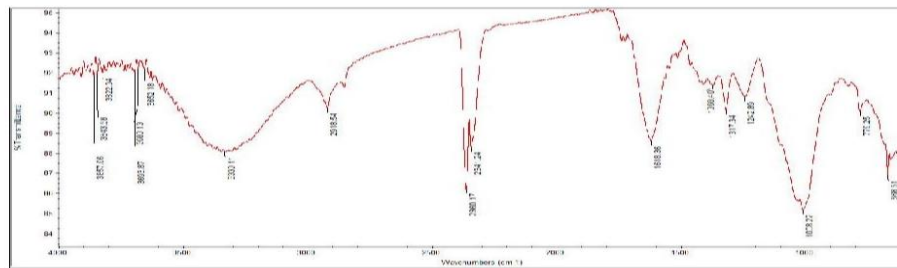
**Table 3:** FT-IR Spectroscopic Data of *A.indicum* Stem and Root

Plant part	Stretching Frequency	Functional Group	Assignments
Stem	779.43	C-Cl stretching	Chloro compounds
	1243.29	$\emptyset$ -O-H asyl- O -stretching	Aromatic ether
	1617.55	NH <sub>3+</sub> asym bending	Amino acid
	2359.78	NH <sub>3+</sub> stretching	Tertiary amino salt
	2918.06	C-H stretching	Cycloalkane
	3311.53	OH stretching	Hydroxyl group
Root	779.25	C-Cl stretching	Chloro compounds
	1212.89	$\emptyset$ -O-H asyl- O -stretching	Aromatic ether
	1618.36	NH <sub>3+</sub> asym bending	Amino acid
	2360.17	NH <sub>3+</sub> stretching	Tertiary amino salt
	2916.54	C-H stretching	Cycloalkane
	3330.11	OH stretching	Hydroxyl group



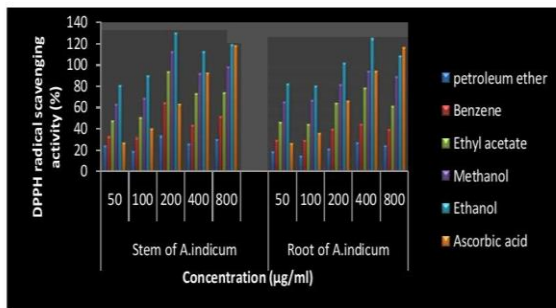


a. FT-IR spectrum of *A.indicum* stem.

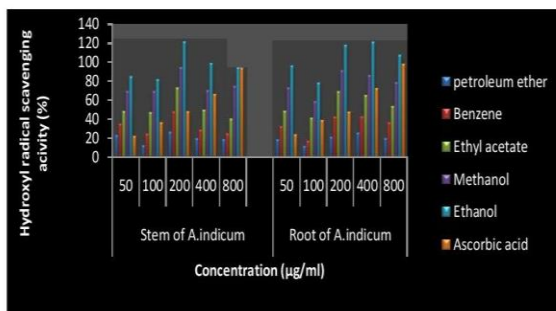


a. FT-IR spectrum of *A.indicum* root.

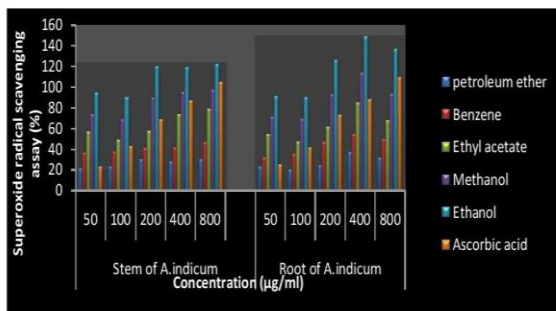
PLATE II



a. DPPH radical scavenging activity of *A.indicum* stem and root

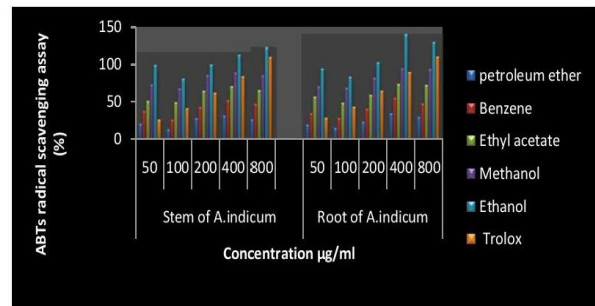


b. Hydroxyl radical scavenging activity of *A.indicum* stem and root

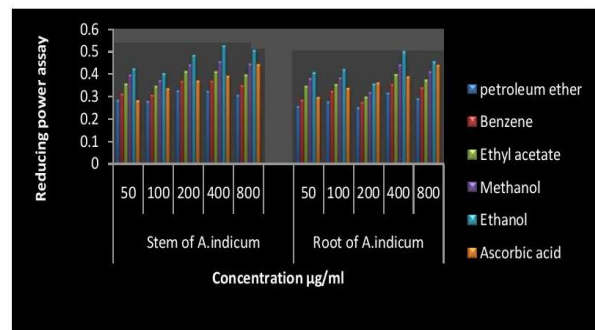


c. Superoxide radical scavenging activity of *A.indicum* stem and root

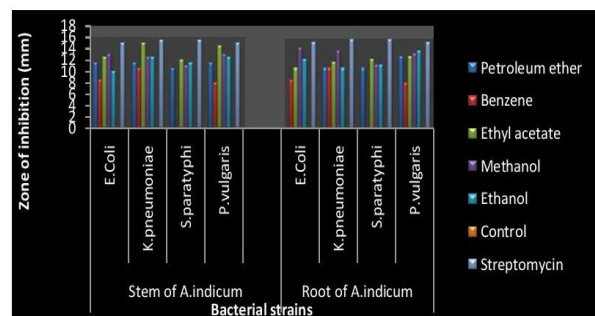
PLATE III



a. ABTs radical scavenging activity of *A.indicum* stem and root



b. Reducing power assay of *A.indicum* stem and root



c. Antibacterial activity of *A.indicum* stem and root

**Table 4:** IC<sub>50</sub> values of Different Solvent Extracts of Stem and Root of *Abutilon indicum*

Solvent	IC <sub>50</sub> (µg/ml)							
	DPPH		Hydroxyl		Superoxide		ABTS	
	Stem	Root	Stem	Root	Stem	Root	Stem	Root
Petroleum ether	28.16	26.15	28.18	28.16	34.16	29.16	34.96	28.16
Benzene	30.16	24.16	27.26	21.65	32.54	28.26	26.18	26.84
\Ethyl acetate	30.92	31.46	31.81	35.13	32.26	41.26	29.88	30.16
Methanol	46.18	37.11	35.16	36.12	46.16	45.12	32.13	40.15
Ethanol	43.16	34.24	39.84	31.26	43.11	42.16	34.18	37.16
Ascorbic acid	32.16	32.16	29.16	29.16	39.81	39.81	-	-
Trolox	-	-	-	-	-	-	31.16	31.16

### **In vitro Antioxidant Activity**

#### **DPPH Radical Scavenging Activity**

The result of DPPH radical scavenging activity of *A.indicum* of stem and root were shown in plate II a. *In vitro* antioxidant activity of the five extracts, the extent of DPPH radical scavenging at different concentrations (50-800 µg/ml) of *A.indicum* stem and root extracts were measured, with ascorbic acid as the standard. *A.indicum* stem and root were showed highest DPPH scavenging activity at increasing concentrations. Methanol extracts of stem and root showed maximum activity with values of 146.88% and 124.33% respectively with IC<sub>50</sub> value 46.18 µg/ml and 37.11 µg/ml respectively (Table 4).

#### **Hydroxyl Radical Scavenging Activity**

*In vitro* antioxidant activity of the five extracts, the extent of hydroxyl radical scavenging at different concentrations (50-800 µg/ml) of *A.indicum* stem and root extracts were measured with ascorbic acid as the standard (Plate II b). The control and the plant extracts showed their maximum activity at increasing concentration. Ethanol and methanol extract of *A.indicum* stem showed highest radical activity with values of 134.88% and 118.46% respectively followed by ethyl acetate and control (ascorbic acid) (99.33% and 98.11%) with IC<sub>50</sub> values: ethanol (39.84 µg/ml), methanol (35.16 µg/ml), ethyl acetate (31.81 µg/ml) and standard (29.16 µg/ml) respectively. Similarly methanol extract of root showed highest radical scavenging activity (121.65%) with IC<sub>50</sub> value 36.12 µg/ml (Table 4).

#### **Superoxide Radical Scavenging Activity**

Petroleum ether extract of stem showed highest superoxide radical scavenging activity (129.16%) followed by ethanol (124.16%), methanol (121.16%) and control (ascorbic acid) (109.81%) with IC<sub>50</sub> values of petroleum ether (34.96 µg/ml), ethanol (34.18 µg/ml), methanol (32.13 µg/ml) and control (31.16 µg/ml). Similarly methanol extract of root showed highest radical scavenging activity (148.34%) followed by ethanol (136.80%), ethyl acetate (126.16%) and control (ascorbic

acid) (109.26%) with IC<sub>50</sub> value of methanol (45.12 µg/ml), ethanol (42.16 µg/ml), ethyl acetate (41.26 µg/ml) and control (139.81 µg/ml) (plate II c) (Table 4).

#### **ABTS Scavenging Assay**

*A.indicum* stem and root extracts were subjected to ABTS radical cation scavenging assay and the results were shown in plate III a. Methanol extracts of stem and root showed highest ABTS scavenging activity (136.86% and 139.16%) with IC<sub>50</sub> value (46.16 µg/ml) and (40.15 µg/ml) and control (trolox) (109.81%) with IC<sub>50</sub> value (31.16 µg/ml) respectively (Table 4).

#### **Reducing Power Assay**

Different solvent extracts of *A.indicum* stem and root showed highest reducing power assay compared to ascorbic acid (Plate III b).

The higher absorbance indicates a higher reducing power. Among the solvent tested methanol and ethanol extract of both stem and root exhibited higher reducing activity compared to control (ascorbic acid).

#### **Antibacterial Activity**

The antibacterial activity of *A.indicum* (stem and root) using five different solvents (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were tested against four human bacterial pathogens viz *E.coli*, *K.pneumoniae*, *S.paratyphi* and *P.vulgaris* were presented in Plate III c.

The maximum inhibition zone was recorded in ethyl acetate extract of *A.indicum* stem against *K.pneumoniae* and *P.vulgaris* and followed by methanol extract. The highest inhibition zone was recorded in methanol extract of *A.indicum* root against *E.coli* and *K.pneumoniae* followed by ethyl acetate. Benzene extracts of *A.indicum* stem and root were active only against three pathogens ie. except *S.paratyphi*.

### **DISCUSSION**

Plant materials are rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food.<sup>26</sup> Phenolic compounds are



considered secondary metabolites and these phytochemical compounds derived from phenylalanine and tyrosine occur ubiquitously in plants and are diversified.<sup>27</sup> Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability.

Phenolics compounds of plants fall into several categories: chief among these are the flavonoids which have potent antioxidant activities. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial, antiviral, antiinflammatory, anticancer and antiallergic activities.<sup>28,29</sup> Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen and various free radicals implicated in several diseases.<sup>30</sup> So comparable with the findings in the literature for other extracts of plant products.<sup>31</sup> In the present results suggested that phenolic and flavonoids may be the major contributors for the antioxidant activity.

FT-IR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation. Results of FT-IR spectroscopic studies have revealed the presence of various chemical constituents of *A.indicum* stem powder with various peak values corresponds to 3311.53, 2918.06, 2359.78, 1617.55, 1243.29 and 779.43  $\text{cm}^{-1}$  stretching frequency. Similarly *A.indicum* root powder have various peak values corresponds to 3330.11, 2916.54, 2360.17, 1618.36, 1212.89 and 779.25  $\text{cm}^{-1}$  stretching frequency. *A.indicum* stem and root have IR stretching frequency at 3311.53 and 3330.11  $\text{cm}^{-1}$  are due to the hydroxyl (acid) stretching frequency. A peak at 2918.06 and 2916.54  $\text{cm}^{-1}$  are to assign C-H asymmetric stretching which is primarily associated with alkyl group. A peak at 2359.78 and 2360.17  $\text{cm}^{-1}$  confirm the presence of tertiary amine salt. The band of 1617.55 and 1618.36  $\text{cm}^{-1}$  indicate the presence of amino acids.

The intense bands occurring at 1243.29 and 1212.89  $\text{cm}^{-1}$  stretching indicate the presence of aromatic ether. A peak at 779.43 and 779.25  $\text{cm}^{-1}$  are to assign C-Cl stretching which is primarily associated with chloro compounds. The FT-IR spectrum confirmed the presence of hydroxyl group, cyclo alkanes, tertiary amino salt, amino acid, aromatic ether and chloro compounds in stem and root of *A.indicum*. Thus, the present study on *A.indicum* stem and root were exhibited novel phytochemical markers as useful analytical tool to check not only the quality of the powder but also the presence of adulterants in pharmaceutical industry.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet colour) and

convert it to yellow coloured  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazine. The degree of decolouration indicates the radical scavenging potential of the antioxidants.<sup>32</sup> In the present investigation, methanol extracts of stem and root of *A. indicum* showed maximum DPPH radical scavenging activity of 146.88% and 124.33% at 800  $\mu\text{g/ml}$  respectively. The values are also comparable in the standard antioxidant ascorbic acid at the same concentration. This suggests that *A. indicum* contain phytochemicals such as phenolics that can donate electron/hydrogen easily.

The hydrogen radical is one of the representative reactive oxygen species generated in the body. These radicals are produced through various biological reactions; one of the common reaction is the Iron (II) based Fenton reaction. *A. indicum* stem and root extracts were found to be effective in hydroxyl radicals scavenging activity and the increase was concentration dependent. Methanol extracts of stem and root of *A. indicum* exhibited highest hydroxyl radical scavenging activity with  $\text{IC}_{50}$  value 35.16  $\mu\text{g/ml}$  and 36.12  $\mu\text{g/ml}$  respectively.

Superoxide anion is a precursor to active free radicals and plays an important role in the formation of the ROS, such as hydrogen peroxide, hydrogen radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA.<sup>33</sup> Superoxide anion derived from dissolved oxygen riboflavin/methionine illuminate system and reduces NBT in this system. In this method, superoxide anion reduces the yellow dye ( $\text{NBT}^{2+}$ ) to produce the blue formation which is measured spectrophotometrically at 560 nm. Antioxidants are able to inhibit the blue NBT formation<sup>34</sup> and the decrease of absorbance at 560 nm indicates the consumption of superoxide anion in the reaction mixture. Among the five solvent tested, petroleum ether extract of stem and methanol extract *A. indicum* root exhibited maximum superoxide radical scavenging activity.

ABTS radical scavenging assay involves a method that generates a blue/green ABTS + chromophore via the reaction of ABTS and potassium persulfate. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 745 nm. The presence of specific chemical compounds in the extracts of *A.indicum* stem and root may inhibit the potassium persulfate activity and hence reduced the production of ABTS. This study reports that the methanol stem and root extracts of *A.indicum* have highest antioxidant activity.

In reducing power assay, the yellow colour of the test solution changes to green depending on the reducing power of the test specimen. The presence of the reductants in the solution causes the reduction of the  $\text{Fe}^{3+}$ /ferricyanide complex to the ferrous form. Therefore,  $\text{Fe}^{2+}$  can be monitored by absorbance measurement at 700 nm. Previous reports suggested that the reducing properties have been shown to exert antioxidant action



by donating of a hydrogen atom to break the free radical chain.<sup>35</sup> In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of *A.indicum* stem and root extracts in concentration dependant manner when compared to the standard ascorbic acid.

In the present study among the five solvent extracts studied ethyl acetate and methanol extracts had moderate activity against the colonial growth of bacteria. The antibacterial properties exhibited by the stem and root extracts of *A.indicum* may be associated with presence of more variety of phytochemicals like saponins, glycosides, steroids, flavonoids, phenols and tannins. The present study confirms that stem and root extracts of *A.indicum* have significant antibacterial activity along with valuable phytochemicals.

### CONCLUSION

In conclusion, the results of the study clearly indicate that methanol and ethanol extracts of *A.indicum* stem and root possess powerful *in vitro* antioxidant activity. The overall antioxidant activity of *A.indicum* might be attributed to its polyphenolic content and other phytochemical constituents. The plant merits further investigation in animal models to confirm its antioxidant activity *in vivo* and to isolate the active constituents, which may result in a modern drug from this plant.

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