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**ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *Andrographis
echioides* (L.) Nees LEAVES EXTRACTS**

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ABSTRACT

The objectives of this study were to determine antimicrobial and antioxidant activities of methanolic leaves extracts of *Andrographis echioides* (L.) Nees. Antimicrobial activity was examined against four gram positive, four gram negative bacterial and two fungal strains. The methanolic leaves extracts was subjected to assess their antioxidant potential using various *in vitro* methods such as DPPH, ABTS, FRAP and reducing power assay. The methanolic leaves extract of *Andrographis echioides* (L.) Nees was more effective antioxidant activity compared to standard. Methanolic leaves extracts of *Andrographis echioides* (L.) Nees found to be potentially effective against Gram positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*) gram negative (*Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*) bacteria and fungi (*Aspergillus niger*, *Candida albicans*). The present study concludes the medicinal significance of *Andrographis echioides* (L.) Nees extracts having potential antimicrobial and antioxidant properties which leads to pharmaceutical applications.

**Keywords: *Andrographis echioides* (L.) Nees, antimicrobial, antioxidant, methanolic, leaves
extracts**

INTRODUCTION

Plant leaves have been used as herbal medicine for their healing properties since ancient times. Some bioactive compounds within these plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannin, flavonoid and phenolic compounds [1]. Due to antibacterial and antioxidant capabilities, low toxicity, and the potential to be a cheaper alternative to pricey synthetic medications, global interest in the study of diverse medicinal plants has exploded in recent decades [2]. Antibiotic resistance is a major issue, and certain commercially available antibiotics have been linked to hypersensitivity and allergic reactions in humans. As a result, in the wake of disease resistance, scientists are searching for various naturally available antimicrobial medicines [3, 4]. Plant-based antimicrobials represent a massive untapped source of medications, and further research into them is urgently needed. Plant-derived antimicrobials have significant therapeutic promise. Antimicrobials produced from plants have a long history of offering much-needed new treatments [5]. Plants ability to perform combinatorial chemistry by mixing, matching, and evolving gene products required for secondary

metabolite biosynthetic pathways results in an infinite pool of chemical compounds [6].

Andrographis echinoides (L.) Nees plant are seen mostly in dry places, such as India, Sri Lanka and South Asian countries, and exhibit diuretic [7], analgesic [8], anti-ulcer [9], hepatoprotective [10] and antioxidant [11] activities. Hence the current study was designed to evaluate the antimicrobial and antioxidant activity of methanolic leaves extracts of *Andrographis echinoides* (L.) Nees by using DPPH, ABTS, FRAP and reducing power assay.

Materials and methods

Collection of plant material

The fresh plant of *Andrographis echinoides* (L.) Nees was collected from Thanjavur District, Tamil Nadu, India and taxonomically identified by the Rapinat Herbarium and center for molecular systematic, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The collected plant was further surface sterilized and it was shaded dried. Once completely dried, the plant leaves was grinded using the electronic blender. Plant powder was kept in a tight container until required.

Preparation of plant extracts

About 100-g plant powder was subjected to extraction by a Soxhlet extractor. The extraction was carried out in methanol. A rotary vacuum evaporator was used to concentrate the extracts, and the remaining solvent was evaporated to dryness in a water bath.

Determination of antioxidant activity by using *in-vitro* method

DPPH radical scavenging ability assay

The percentage of antioxidant activity of extracts was assessed by DPPH free radical assay. The sample was reacted with the stable DPPH radical in methanol. 0.3 mM concentration of DPPH standard solution was prepared by dissolving 118.2 g of DPPH (1,1-diphenyl-2-picrylhydrazyl) in 1000 mL of methanol. Sample stock solution was made by dissolving 0.01g in 1 mL of respective solvents (100 mg/mL) and from that different concentration was prepared such as 10, 20, 30, 40 and 50 µg/mL. One milliliter of each sample solution was combined with two milliliters of DPPH reagent and kept in the dark for 30 minutes before reacting at room temperature. When the antioxidant chemicals in plant extracts react with DPPH, the DPPH is reduced and the colour changes from deep violet to bright yellow. After 30 minutes, the absorbance was

recorded at 517 nm in UV-Visible spectrophotometry and the percentage of radical scavenging activity i.e., antioxidant activity was calculated by following standard formula. Control reading was read by adding one milliliter of solvent with two milliliter of DPPH reagent [12, 13].

$$\% \text{ of DPPH Scavenged} =$$

$$\frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

$$\text{Ab of control} = \text{Control Absorbance, Ab of test} = \text{Test solution Absorbance}$$

Linear regression graphs were used to obtain the IC₅₀ value, with the abscissa representing the concentration of the tested sample and the ordinate representing the average percent of radical scavenging activity.

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging assay

The ABTS radical cation decolorization assay was used to determine the plant sample free radical scavenging activity. The reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate (1:1) produced the ABTS^{•+} radical, which was kept in the dark at room temperature for 12-16 hours before use. After diluting the ABTS^{•+} solution with methanol, an absorbance of 0.700 at 734 nm was obtained. The absorbance was measured 30 minutes after the addition of 5 liters of plant extracts

to 3.995 liters of diluted ABTS^{•+} solution.. In each test, a suitable solvent blank was used. All of the tests were repeated at least three times. The formula was used to compute the percent suppression of absorbance at 734 nm. As a standard drug, Trolox was employed [14].

$$\% \text{ of Scavenging Activity} = \frac{\text{Ab of control} - \text{Ab of test}}{\text{Ab of control}} \times 100$$

Ab of control = absorbance of ABTS radical + methanol, Ab of test = absorbance of ABTS radical + sample extract/standard.

Linear regression graphs were used to obtain the IC₅₀ value, with the abscissa representing the concentration of the tested sample and the ordinate representing the average percent of radical scavenging activity.

FRAP (Ferric Reducing/Antioxidant Power) ASSAY

The stock solutions included 300 mM acetate buffer (3.1 g C₂H₃NaO₂. 3H₂O and 16 mL C₂H₄O₂), pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl₃.6H₂O solution. 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL FeCl₃. 6H₂O solution were combined to make the fresh working solution, which was then warmed to 37 °C. For 30 minutes in the dark, different concentrations of extracts (0.15 mL) were allowed to react with 2.80

mL of the FRAP solution. At 593 nm, measurements of the colourful product (ferrous tripyridyl triazine complex) were made. As a standard drug, Trolox was employed [15].

Reducing power assay

Phosphate buffer (2.5 mL) and potassium ferricyanide were combined with various amounts of extracts in matching solvents (2.5 mL). This mixture was maintained in a water bath at 50 °C for 20 minutes. When necessary, 2.5 mL of 10% trichloroacetic acid was added after cooling and centrifuged at 3000 rpm for 10 minutes. The solution was combined with distilled water (2.5 mL) and a freshly made ferric chloride solution in the upper layer (2.5 mL) (0.5 mL). At 700 nm, the absorbance was measured. The control was made in the same way as the samples, but without the samples. As a standard, various amounts of ascorbic acid were utilized. An increase in the reaction mixture's absorbance suggests an increase in the reducing power [16].

Determination of anti-microbial activity

The *in vitro* antimicrobial activity of the methanolic leaves extracts of *Andrographis echinoides* (L.) Nees was assessed by various assays towards pathogenic bacteria and fungi. The bacteria include both gram-positive and gram-

negative bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Streptococcus epidermidis* and *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and fungi are *Aspergillus niger* and *Candida albicans*. Bacterial and fungal strains was maintained on Nutrient agar slants (Hi media) at 4 °C. The diameter of the inhibition zone generated around each disc as a result of disc diffusion of antimicrobial compounds from the paper discs into the surrounding medium was measured. The antibacterial activity of plant extracts was the highest. The diameter of each extract's inhibition zone against each germ was found to be less than that of a conventional antibiotic medication (Gentamicin 10 µg/disc).

RESULTS AND DISCUSSION

Free radical-scavenging ability by the DPPH assay

Medicinal plants play important roles as powerful antioxidants due to the occurrence of polyphenolic compounds in abundance. Phenolic compounds decrease the risk of serious health concerns due to the resistance of the oxidative damage by reactive oxygen species (ROS). Previous studies have been reported antioxidant activity along with antileishmanial activity in which good antileishmanial compounds were

good antioxidant also [17-20]. The DPPH is a constant radical with a highest absorption at 517 nm that can readily undertake scavenging by antioxidant [21]. It has been broadly used to assess the capability of compounds as hydrogen donors or free-radical scavengers and the antioxidant activity of plant extracts [22, 23].

The free radical scavenging activity of *Andrographis echinoides* (L.) Nees leaves extracts was analyzed using DPPH assay. Varying concentrations of the sample was used from 10 to 50 µg/mL. It has been observed that the free radical scavenging activity increases with the increase in concentration of the leaves extracts. It is also observed that the leaves extracts has excellent antioxidant activity showing IC₅₀ value of 15.07 µg/mL compared to standard (Ascorbic acid) 24.36 µg/mL. The antioxidant activity of the leaves extracts are observed in lower IC₅₀. The free radical scavenging activity of *Andrographis echinoides* (L.) Nees leaves extracts is higher activity than standard (Ascorbic acid) in Table-1 and shown in **Figure 1, 2**. The more phenolic compounds are in the plant, the lower IC₅₀ is obtained in the DPPH method.

Free radical-scavenging ability by the ABTS assay

The principle objective of this test is to measure the capacity of different substances to scavenge the ABTS^{•+} radical cation. Antioxidant capacities was expressed by IC₅₀ values, indicating the concentrations of extracts scavenge 50% of ABTS^{•+} radical. The IC₅₀ values of the *Andrographis echinoides* (L.) Nees leaves extracts ABTS^{•+} radical scavenging activity was 104.54 µg/mL and the standard (Trolox) 32.67 µg/mL as shown in **Table 2** and illustrated in **Figure 3, 4**.

Free radical-scavenging ability by the FRAP assay

Reducing power assay is often used to evaluate the ability of antioxidants to donate electron [24, 25]. Ferric reducing antioxidant power (FRAP) assay is a quantitative assay for measuring the antioxidant potential within a sample. Ferric iron (Fe³⁺) is reduced, by electron-donating antioxidants present in the extracts, to its ferrous form (Fe²⁺). Results of reducing power showed that the antioxidants present in the sample reduce the ferric complex Fe³⁺ into ferrous Fe²⁺ form. The reducing power was expressed as IC₅₀ value. The results depicted in **Table 3** and shown in **Figure 5, 6**. It is revealed that methanolic leaves extract of *Andrographis echinoides* (L.) Nees exhibited the potent activity 52.53 µg/mL

and the standard (Trolox) 42.11 µg/mL. Therefore, this study revealed that the high levels of phenolic compounds found in the dominant contributors to the antioxidant activity in the leaves extracts.

Free radical-scavenging ability by the Reducing power assay

The reducing power of *Andrographis echinoides* (L.) Nees leaves extracts was measured, using ascorbic acid as standard. The reducing power assay was measured for different concentrations varying from 10 to 50 µg/mL and the absorbance was recorded. It can be observed that with the increase in the concentration of leaves extracts the reducing activity increases. The results are summarized in **Table 4** and shown in **Figure 7, 8**. The inhibition range of *Andrographis echinoides* (L.) Nees reducing power activity is 192.72 µg/mL at various concentrations (10, 20, 30, 40, and 50 µg/mL), compared to the standard (Ascorbic acid) 49.75 µg/mL. Therefore, it can be predicted that the leaves extracts could be a good reducing power activity.

IC₅₀ value of different radical scavenging tests (DPPH, ABTS, FRAP, Reducing power)

Table 5, below presence the IC₅₀ (50% inhibitory concentration) of the methanolic leaves extracts tested. The results

showed that the IC_{50} varied between 15.07 $\mu\text{g/mL}$ and 192.72 $\mu\text{g/mL}$ depending on the type of radicals. The different concentration of extracts tested for reducing power, DPPH, ABTS, FRAP radicals showed the better IC_{50} compared to the standard. The IC_{50} of the extracts of *Andrographis echinoides* (L.) Nees DPPH assay was lower (15.07 $\mu\text{g/mL}$) than the standard (24.36 $\mu\text{g/mL}$). This observation gives an indication of strong antioxidant potential of the extracts, which is confirmed with DPPH, FRAP, reducing power and ABTS radicals. The present study proved that the extracts have potent antioxidant activity and can be useful for the management of various diseases.

Antimicrobial activity

The methanolic leaves extract of *Andrographis echinoides* (L.) Nees was investigated for their antimicrobial properties against gram positive, gram negative bacteria

and fungi using agar well disc diffusion method. Evaluation of antimicrobial activities of this plant extracts was recorded in **Table 6** and shown in **Figure 9**. When the crude extract was compared with standard antibiotic (Gentamicin), the plant extracts showed varying degree of antimicrobial potential due to different concentration.

Methanolic leaves extracts of *Andrographis echinoides* (L.) Nees found to be potentially effective against gram positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*) gram negative (*Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*) bacteria and fungi (*Aspergillus niger*, *Candida albicans*). The above results indicated that methanolic leaves extracts of *Andrographis echinoides* (L.) Nees exhibited a better antimicrobial activity against gram positive, gram negative bacterial and fungal strains than standard drug.

Table 1: Analysis of DPPH radical scavenging activity of leaves extracts of *Andrographis echinoides* (L.) Nees

S. No	Extract Name	Sample Concentration ($\mu\text{g/mL}$) with respective % of Inhibition					IC_{50} Value $\mu\text{g/mL}$
		10	20	30	40	50	
1	<i>Andrographis echinoides</i> (L.) Nees	44.01 \pm 1.01	56.18 \pm 0.06	69.07 \pm 0.11	80.91 \pm 0.48	95.71 \pm 0.38	15.07
2	Ascorbic acid	29.08 \pm 1.23	47.50 \pm 1.50	58.17 \pm 1.98	69.01 \pm 2.05	78.14 \pm 2.97	24.36

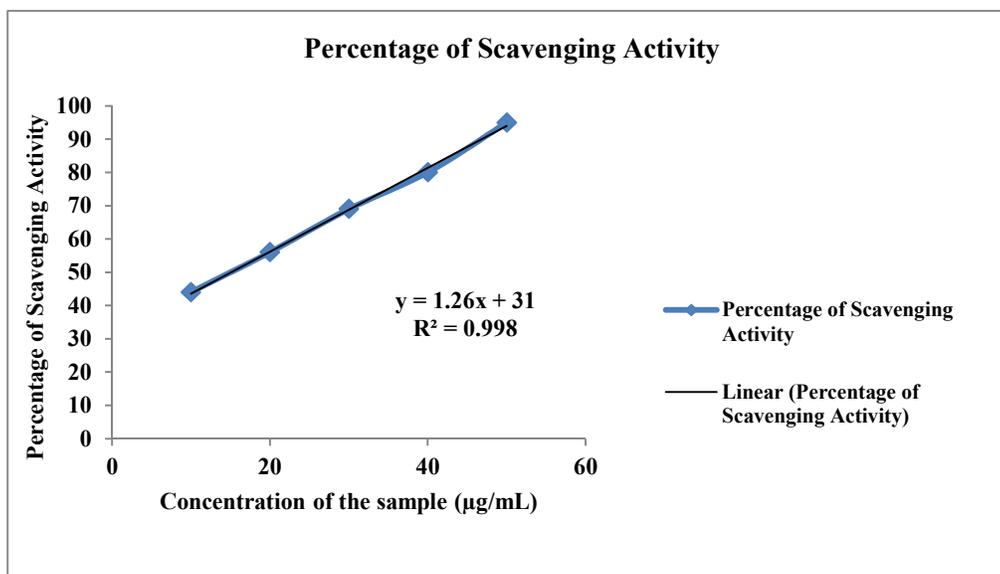


Figure 1: DPPH radical scavenging activity of the extracts of *Andrographis echioides* (L.) Nees

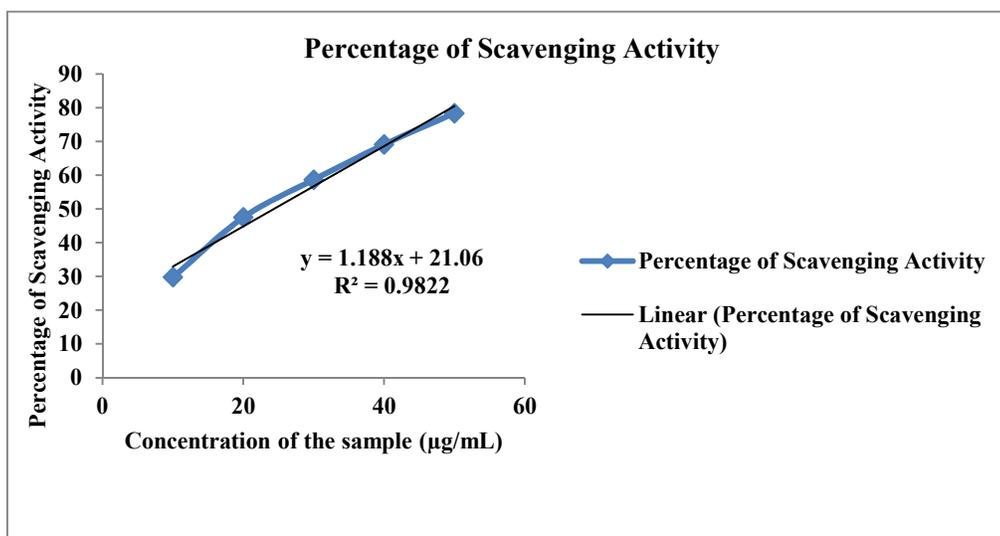


Figure 2: DPPH radical scavenging activity of standard (Ascorbic acid)

Table 2: Analysis of ABTS radical scavenging activity of leaves extracts of *Andrographis echioides* (L.) Nees

S. No	Extract Name	Sample Concentration (µg/mL) with respective % of Inhibition					IC ₅₀ Value µg/mL
		10	20	30	40	50	
1	<i>Andrographis echioides</i> (L.) Nees	19.27±0.06	22.48±0.01	25.68±0.01	29.96±0.05	32.26±0.01	104.54
2	Trolox	23.8±0.43	37.5±0.50	48.7±0.90	53.1±1.05	68.4±1.07	32.67

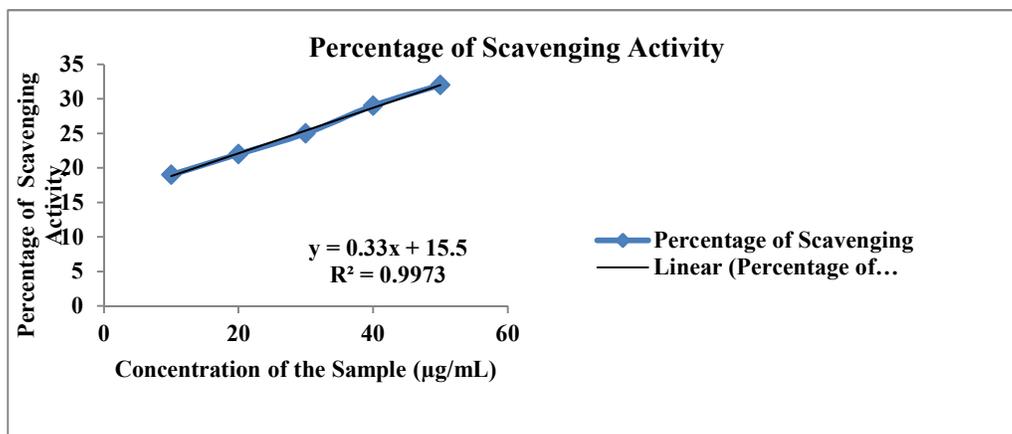


Figure 3: ABTS radical scavenging activity of the extracts of *Andrographis echinoides (L.) Nees*

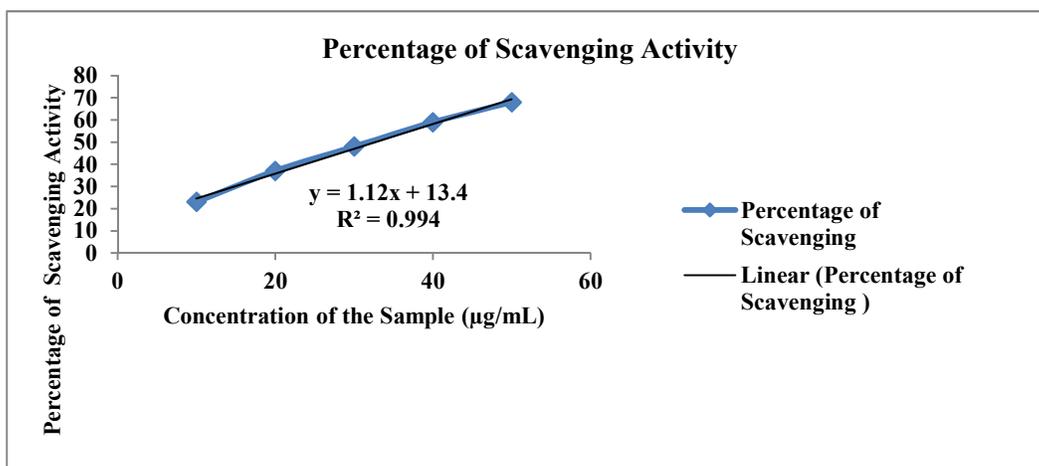


Figure 4: ABTS radical scavenging activity of standard (Trolox)

Table 3: Analysis of FRAP radical scavenging activity of leaves extracts of *Andrographis echinoides (L.) Nees*

S. No.	Extract Name	Sample Concentration (µg/mL) with respective % of Inhibition					IC ₅₀ Value µg/mL
		10	20	30	40	50	
1	<i>Andrographis echinoides (L.) Nees</i>	16.2±0.04	24.8±0.04	33.1±0.00	41.1±0.00	47.9±0.00	52.53
2	Trolox	27.60±0.01	34.01±0.11	42.72±0.04	49.09±0.13	55.06±0.12	42.11

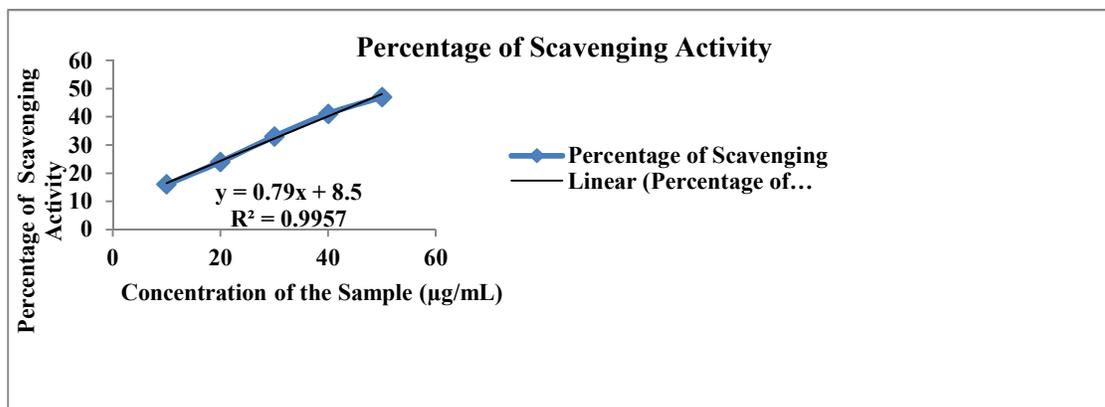


Figure 5: FRAP (Ferric Reducing/Antioxidant Power) assay of the extracts of *Andrographis echinoides (L.) Nees*

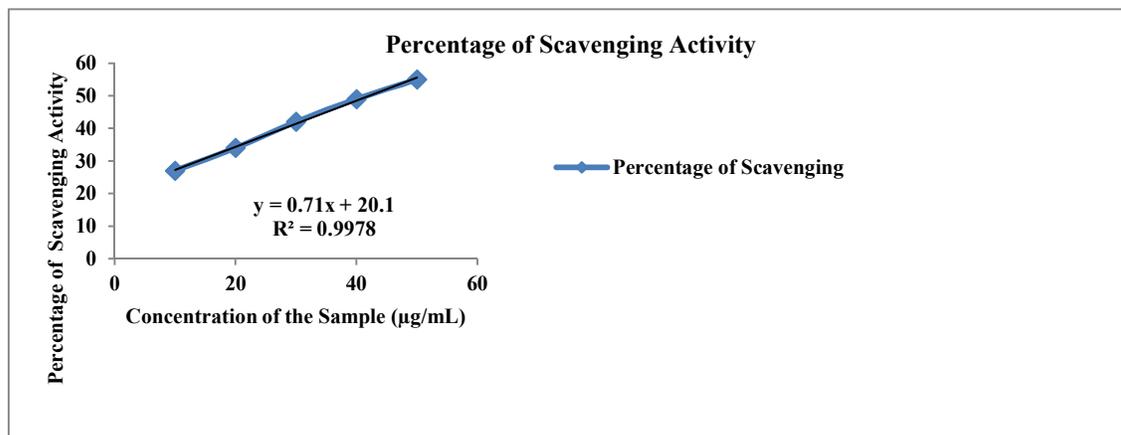


Figure 6: FRAP radical scavenging activity of standard (Trolox)

Table 4: Analysis of reducing power assay of leaves extracts of *Andrographis echioides* (L.) Nees

S. No.	Extract Name	Sample Concentration (µg/ml) with respective % of Inhibition					IC ₅₀ Value µg/ml
		10	20	30	40	50	
1	<i>Andrographis echioides</i> (L.) Nees	10.9±0.00	12.4±0.00	14.3±0.01	16.6±0.01	19.9±0.01	192.72
2	Ascorbic acid	25.80±0.03	30.11±0.10	41.02±0.14	46.18±0.03	50.02±0.01	49.75

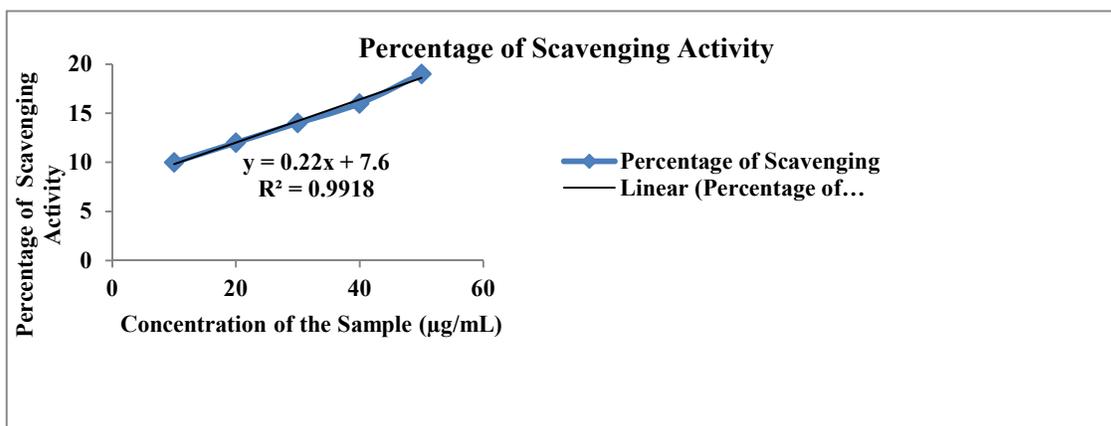


Figure 7: Reducing power activity of the extracts of *Andrographis echioides* (L.) Nees

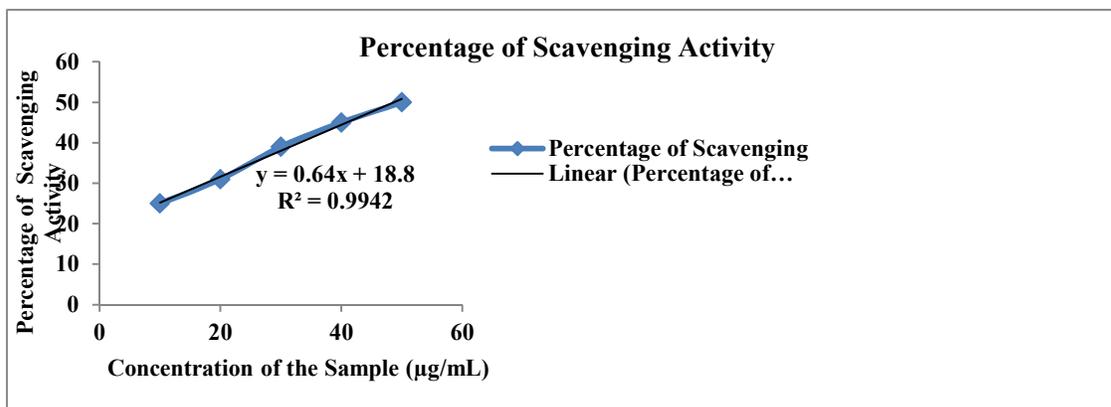


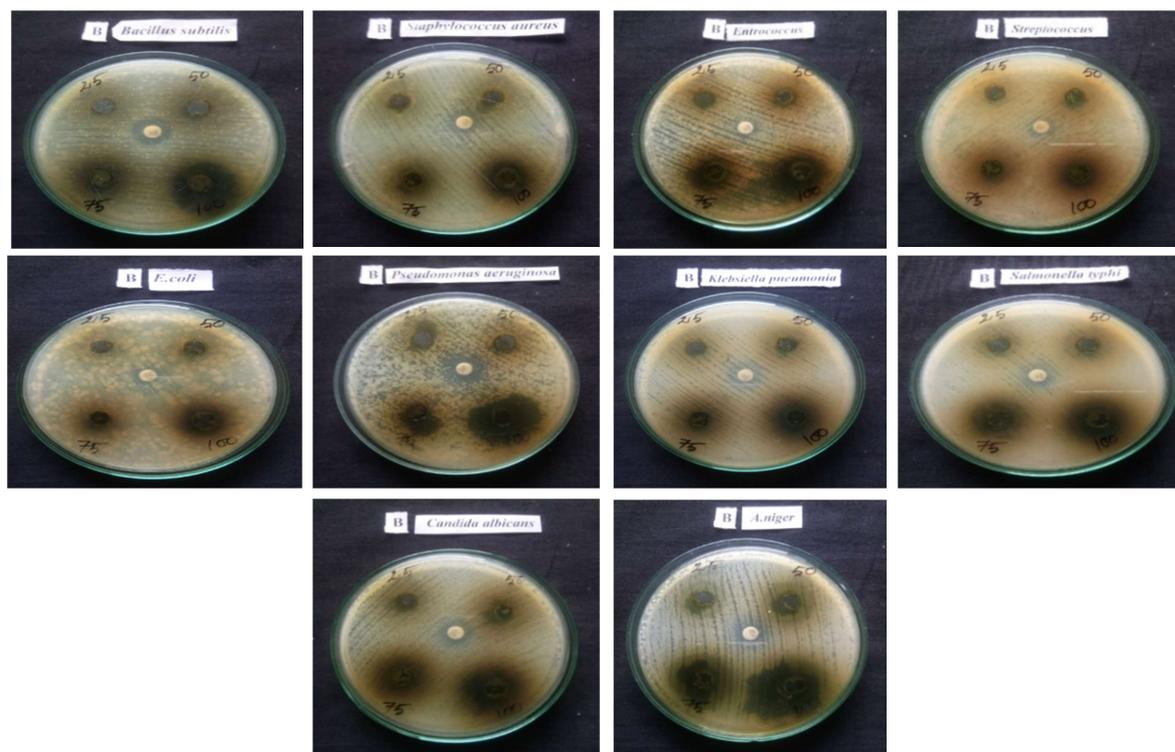
Figure 8: Reducing power activity of standard (Ascorbic acid)

Table 5: Fifty percentage inhibitory concentration (IC₅₀) values of *Andrographis echinoides* (L.) Nees

S. No.	Extract Name	IC ₅₀ Value µg/mL			
		DPPH	ABTS	FRAP	Reducing power
1	<i>A.echinoides</i> (L.) Nees	15.07	104.54	52.53	192.72
2	Standard	24.36	32.67	42.11	49.75

Table 6: Antimicrobial activity of *A. echinoides* (L.) Nees

S.No.	Name of organisms	Inhibition values in mm				
		25 µl	50 µl	75 µl	100 µl	Control
1	<i>Bacillus subtilis</i>	20	23	26	30	20
2	<i>Staphylococcus aureus</i>	20	23	26	28	20
3	<i>Enterococcus faecalis</i>	20	24	26	29	18
4	<i>Streptococcus epidermidis</i>	18	22	24	26	20
5	<i>E. coli</i>	20	24	26	28	20
6	<i>Pseudomonas aeruginosa</i>	20	24	27	30	20
7	<i>Klebsiella pneumonia</i>	20	22	24	27	18
8	<i>Salmonella typhi</i>	18	21	23	26	20
9	<i>Candida albicans</i>	20	24	26	29	20
10	<i>Aspergillus niger</i>	20	25	30	35	22

Figure 9: Antimicrobial activity of *A. echinoides* (L.) Nees leaves extracts

CONCLUSION

The present study describes antimicrobial activities of methanolic leaves extracts of *Andrographis echinoides* (L.) Nees which shows higher activity against the tested bacteria and fungi. The methanolic leaves extract of *Andrographis echinoides* (L.) Nees exhibits better antioxidant activity than standard drug. These results encourage complementary studies on the chemical composition of the plant with aim of the separation and structural characterization of their active compounds.

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