



Antioxidant Potentialities & Phytochemical Studies of *Ageratina Adenophora*, *Sonchus Wightianus* Dc, *Artemisia Nilagirica* Roots, Stems and Leaves Using Neomycin and Miconazole Drug

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ABSTRACT:

In the present study, the antimicrobial and antioxidant properties. They have the remarkable ability to enhance both humoral (related to bodily fluids) and cell-mediated immune responses due to the various compounds they contain. In the pharmaceutical industry, many plant-derived phytochemicals are used as antimicrobial agents to effectively combat serious infectious diseases caused by bacteria, fungi, and viruses without causing harmful side effects. Additionally, plant-based antioxidants, whether in their natural form or as chemical components, are highly efficient in protecting against the damaging effects of oxidative stress. Some natural antioxidants found in plants prevent the formation of reactive oxygen species (ROS), while others scavenge free radicals and bind to transition metals.

In this study, the researchers analyzed the methanol, ethanol, and aqueous extracts of three different parts (*A. adenophora*, *A. nilagirica*, and *S. wightianus*) using gas chromatography and mass spectrometry (GC-MS). The analysis focused on identifying the presence of specific phytochemical groups, such as alkaloids, flavonoids, glycosides, terpenoids, phenols, and steroids, to assess their contribution to the antimicrobial and antioxidant potential of these plants. Previous research suggested that a high content of total phenols and total flavonoids might be responsible for the strong antioxidant potential observed in plant extracts. It's important to note that different solvents can affect the efficiency of extracting bioactive compounds from plants. Surprisingly, despite their potential, the chemical analysis of these particular invasive plants had not been investigated in the available literature.

Keywords: *adenophora*, *A. Nilagirica*, *S. Wightianus*, antioxidant potentialities , phytochemical studies

1. INTRODUCTION:

Plants have played a crucial role in human culture and civilization for a very long time. They have been used for various purposes, such as traditional medicines, flavoring food, preserving food, and preventing diseases. The special chemicals in plants, called secondary metabolites, are responsible for their unique properties. These plant-derived substances also help control the growth of microorganisms in different situations. Phytochemicals are natural chemical compounds found in plants, like fruits, vegetables, whole grains, beans, nuts, and seeds. These substances can act as antioxidants, which help protect our bodies from harm. Some well-known antioxidants include vitamins A, C, and E, as well as phenolic compounds like flavonoids, tannins, and lignins found in plants. Different parts of plants, like leaves, roots, or fruits, can contain various types of phytochemicals. These plant compounds are getting a lot of attention because they have different beneficial effects on our health.^{1,2}

There are two main categories of plant chemicals: primary and secondary metabolites. Primary metabolites like proteins, fats, and sugars are found in all plants. Secondary metabolites, such as phenols, flavonoids, terpenoids, glycosides, and alkaloids, are found in smaller amounts but often have strong antioxidant properties and can provide various health benefits. Antioxidants are important because they help protect our bodies from oxidative stress, which can damage our cells and lead to health problems like heart diseases, diabetes, cancer, and other illnesses. Antioxidant phytochemicals are essential in controlling oxidative stress and preventing issues like premature aging, diabetes, cancer, and degenerative diseases. Researchers are increasingly interested in exploring how plant-based products can be used as antioxidants to promote better health.³

2. MATERIALS AND METHOD :

Chemical profiling via GC-MS analysis: A process of discovering and measuring the presence of secondary metabolites in plant extracts was conducted using Gas Chromatography coupled with Mass Spectrometry (GC-MS). Here's how it was done:

- 1. Instrumentation:** The analysis was performed using a Shimadzu QP2010 Plus GC-MS system. This system included an Rtx-5 MS capillary column with specific dimensions (0.25 mm internal diameter and 30 mm in length) made by J&W Scientific (Agilent, Santa Clara, CA, USA).⁴
- 2. Carrier Gas:** Helium gas was used as the carrier gas, and it flowed through the column at a rate of 1.21 ml/min during the analysis.
- 3. Temperature Program:** The oven temperature was controlled in a specific manner. It began at 100°C for the first 2 minutes, then increased to 250°C at a rate of 5°C per minute, and finally to 280°C at a faster rate of 10°C per minute.
- 4. Sample Injection:** One micro liter (1 µl) of the plant extract sample was injected into the column. The injection was done in split mode with a split ratio of 10.
- 5. Detection and Identification:** The GC-MS system used a Mass Spectrometry (MS) detector in full scan mode to detect the presence of various metabolites. By analyzing the distinctive fragmentation patterns (mass spectra) of different compounds, the components in the plant extracts were identified.⁵
- 6. Comparison with Libraries:** To confirm the identity of the components, their spectral data were compared with the standard mass spectra stored in libraries such as the NIST-MS (National Institute of Standards and Technology Mass Spectral Library) and Wiley library. This comparison helped in identifying and quantifying the specific secondary metabolites present in the plant extracts.⁶

This process allowed researchers to identify and measure the secondary metabolites within the plant extracts using GC-MS, which is a powerful analytical technique for this purpose.

Evaluation of antioxidant activity:

Radical scavenging activity by the DPPH Assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging ability of different extracts of selected plants or plant parts was assessed using a method based on the protocol described by Xie et al. (2010) with some modifications. Here's how the experiment was carried out:

DPPH Solution Preparation: A fresh solution of DPPH was prepared at a concentration of 0.1 millimolar (mM). This solution was made in methanol.

Sample and Control Mixtures: Two milliliters (2 ml) of the DPPH solution were mixed with 2 ml of the plant extract. The plant extract was prepared at different concentrations ranging from 10 to 100 micrograms per milliliter (µg/ml). The purpose of this step was to test the scavenging ability of the plant extracts against the DPPH radical at various concentrations.⁷

Control Group: As a control, a mixture of 2 ml of DPPH in 2 ml of methanol (without the plant extract) was used. This control represents the reaction of DPPH without the influence of the plant extract.

Incubation: The reaction mixture, which includes the DPPH solution and the plant extract or control solutions, was incubated in darkness for 30 minutes at room temperature. During this incubation period, the plant extract had the opportunity to scavenge the DPPH radicals.⁸

Spectrophotometric Analysis: After the 30-minute incubation, the absorbance of the reaction mixtures was measured spectrophotometrically at a specific wavelength of 517 nanometers (nm). This measurement provides information about the amount of DPPH radicals remaining in the solution. A lower absorbance indicates a higher level of DPPH scavenging, which is a sign of antioxidant activity.

Positive Control: Ascorbic acid was used as the positive control in the experiment. This compound is known for its antioxidant properties and was likely used to compare the scavenging ability of the plant extracts to a well-established antioxidant.⁹

Calculation of Free Radical Scavenging Potential: The free radical scavenging potential of the plant extract was calculated using a formula described by Blois in 1958. This formula takes into account the change in absorbance caused by the plant extract and provides a percentage that represents the ability of the extract to scavenge the DPPH radicals.

In summary, this experiment aimed to evaluate the antioxidant or free radical scavenging potential of plant extracts by measuring their ability to reduce DPPH radicals. The results were compared to a positive control (ascorbic acid) to assess the efficacy of the plant extracts as antioxidants.¹⁰

Calculated with the formula following:

Inhibition % = $\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$

Absorbance of control * 100

The IC₅₀ value of the test sample and standard was calculated from the graph of inhibition percentage plotted against concentration.

FRAP Antioxidant Assay: The Ferric Reducing Antioxidant Power (FRAP) assay was conducted following the method described by Shahwar et al. (2012) with some minor modifications. This assay assesses the reductive potential of an extract based on its ability to chemically reduce Fe³⁺ to Fe²⁺. Here's a breakdown of the procedure:

Sample Preparation: Ten microliters (10 µl) of the sample extract were taken and diluted to a final volume of 100 µl with double-distilled water. This dilution step is important to prepare the sample for the FRAP assay.

FRAP Reagent Preparation: A freshly prepared FRAP reagent was used. The FRAP reagent consists of 300 mM acetate buffer with a pH of 3.6, 10 mM tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₃•6H₂O in the ratio of 10:1:1. This reagent is important for the redox reaction that will take place.

Sample Mixing: The 100 µl of diluted sample extract was mixed with 1.5 ml of the freshly prepared and pre-warmed (at 37°C) FRAP reagent. This mixture allows the reductive reaction to occur between the sample and the Fe³⁺ ions.

Incubation: The sample and FRAP reagent mixture was kept at 37°C for a duration of 10 minutes. During this incubation period, the reductive reaction occurred, leading to the reduction of Fe³⁺ to Fe²⁺.

Absorbance Measurement: After the 10-minute incubation, the absorbance of the reaction mixture was measured at a specific wavelength of 593 nm using a spectrophotometer. This measurement provides information about the ability of the sample to reduce Fe³⁺ ions, which is indicative of its antioxidant capacity.

Standard Control: Ascorbic acid was used as a standard control. Ascorbic acid is known for its antioxidant properties and was likely used to provide a reference point for evaluating the antioxidant potential of the sample.

Calculation of Reductive Potential: The reductive potential (%) of the plant extract was calculated using a formula provided by Blois in 1958. This formula takes into account the change in absorbance caused by the sample extract and provides a percentage that represents the reductive potential of the extract, which is indicative of its antioxidant activity.¹³

In summary, the FRAP assay is a common method to assess the antioxidant capacity of a sample by measuring its ability to reduce Fe³⁺ to Fe²⁺. The results were compared to a standard control (ascorbic acid) to evaluate the antioxidant potential of the plant extract.

MCA antioxidant Assay: The Metal-Chelating Antioxidant Assay was carried out based on the method described by Chew et al. (2009) with some minor modifications. This assay assesses the chelating activity of a sample, which involves the ability to form complexes with metal ions, thus preventing them from participating in oxidative reactions. Here's how the assay was conducted:¹⁴

Reagent Preparation: To perform this assay, a 0.1 mM solution of FeSO₄ (ferrous sulfate) and a 0.25 mM solution of ferrozine were prepared. Ferrozine is a compound that forms a complex with Fe²⁺ ions.

Sample and Control Mixtures:

For the test samples, 0.2 ml of the plant extract was used.

For the control, 1 ml of distilled water was mixed with 1 ml of the 0.1 mM FeSO₄ solution and 1 ml of the 0.25 mM ferrozine solution. This control represents a situation where the chelation of Fe²⁺ ions does not occur, serving as a reference point.

Complex Formation: In the test samples, the 0.2 ml of plant extract was combined with the 0.1 mM FeSO₄ and 0.25 mM ferrozine solutions. This step is essential to determine the ability of the plant extract to chelate Fe²⁺ ions.

Incubation: Both the test samples and the control mixture were incubated at room temperature for a period of 10 minutes. During this incubation, any chelation between the plant extract and Fe²⁺ ions could take place.¹⁴

Absorbance Measurement: After the 10-minute incubation, the absorbance of the mixture in both the test samples and the control was recorded at a wavelength of 562 nm using a spectrophotometer. This measurement indicates the extent of complex formation, which reflects the chelating potential of the plant extract.

Standard Control: In this experiment, EDTA (Ethylene diamine tetraacetic acid) was used as a standard control. EDTA is a well-known chelating agent used for reference purposes.

Calculation of Chelating Potential: The chelating potential (%) of the plant extract was calculated using a formula, likely similar to the one described by Blois in 1958. This formula takes into account the change in absorbance caused by the sample extract and provides a percentage that represents the chelating potential, indicative of its antioxidant activity.

In summary, the Metal-Chelating Antioxidant Assay measures the ability of a sample to chelate or bind metal ions, such as Fe²⁺, thus preventing them from participating in oxidative reactions. This ability is a key component of the sample's antioxidant activity. Results are compared to a standard control (EDTA) for evaluating the chelating potential of the plant extract.^{15:16}

3. RESULTS AND DISCUSSION:

Ageratina adenophora Spreng :To assess the antimicrobial and antioxidant potential of different parts of *A. adenophora* using various solvent extracts, a total of 9 extracts were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). These extracts were obtained from the leaves, stem, and root parts of the plant using different solvents. Here's a general overview of the process:

Chemical characterization of different Solvent extracts of *A.adenophora* leaves, stem and root:

Methanol Extract: The GC-MS analysis of the methanol extracts from the leaves, stem, and root parts of *A. adenophora* revealed the presence of various phytoconstituents. The analysis identified the ten major compounds in each of these extracts. Here is a summary of the major compounds and their respective maximum concentrations, along with their molecular formulas and molecular weights:

Leaf Extract: Maximum concentration: 2(1H) Naphthalene, 3,5,6,7,8,8a-hexahydro-4,8a-Dimethyl-6-(1-Methylethenyl) (25.09%)

- Molecular formula: C₁₆H₂₄O
- Molecular weight: 232.36 g/mol

Stem Extract:

- Maximum concentration: Guanosine (30.18%)
- Molecular formula: C₁₀H₁₃N₅O₅
- Molecular weight: 283.24 g/mol

Root Extract:

- Maximum concentration: Lupa-13(18),20(30)-Dien-3-yl-acetate (12.23%)
- Molecular formula: C₃₀H₄₆O₂
- Molecular weight: 438.68 g/mol

These major compounds represent the most abundant constituents in each part of the plant extract. The molecular formula and weight of each compound provide information about their chemical structure and mass. The presence of these compounds in the extracts may contribute to the plant's potential antimicrobial and antioxidant properties, as observed in the earlier analysis. These compounds can have various biological activities and may have significance in the context of medicinal or pharmaceutical research.

Ethanol extract: The GC-MS analysis of the ethanol extracts from the leaves, stem, and root parts of *A. adenophora* revealed the presence of various phytoconstituents. The analysis identified the ten major compounds in each of these extracts. Here is a summary of the major compounds and their respective maximum concentrations, along with their molecular formulas and molecular weights:

Leaf Extract:

- Maximum concentration: Delta.9-Capnellene-2-Epsilon-ol-8-one (29.25%)
- Molecular formula: C₁₅H₂₂O₂
- Molecular weight: 234.33 g/mol

Stem Extract:

- Maximum concentration: 24(S) Ethyl-3 Alpha,5 Alpha-cyclocholest- 22(E)-en-6-one (15.96%)
- Molecular formula: C₃₀H₄₈O
- Molecular weight: 424.70 g/mol

Root Extract:

- Maximum concentration: Lupa-13(18),20(30)-Dien-3-yl-acetate (12.23%)
- Molecular formula: C₃₀H₄₆O₂
- Molecular weight: 438.68 g/mol

These major compounds represent the most abundant constituents in each part of the plant extract when using ethanol as the solvent. The molecular formula and weight of each compound provide information about their chemical structure and mass. These compounds may contribute to the plant's potential antimicrobial and antioxidant properties. They could also be of interest in medicinal or pharmaceutical research due to their biological activities.

3. Aqueous extract: The GC-MS analysis of the aqueous extracts from the leaves, stem, and root parts of *A. adenophora* revealed the presence of various phytoconstituents. The analysis identified the ten major compounds in each of these extracts. Here is a summary of the major compounds and their respective maximum concentrations, along with their molecular formulas and molecular weights:

Leaf Extract:

- Maximum concentration: 1-(4-Isopropylphenyl)-2-Methylpropyl Acetate (15.47%)
- Molecular formula: C₁₄H₂₀O₂

- Molecular weight: 220.31 g/mol

Stem Extract:

- Maximum concentration: 1,3,4,5-Tetrahydroxy cyclohexane carboxylic acid (17.89%)
- Molecular formula: C₇H₁₂O₆
- Molecular weight: 192.17 g/mol

Root Extract:

- Maximum concentration: Tetradecanol (19.37%)
- Molecular formula: C₁₄H₃₀O
- Molecular weight: 214.38 g/mol

These major compounds represent the most abundant constituents in each part of the plant extract when using water (aqueous extract) as the solvent. The molecular formula and weight of each compound provide information about their chemical structure and mass. These compounds may contribute to the plant's potential antimicrobial and antioxidant properties. Their identification through GC-MS analysis can be valuable for understanding the chemical composition of the plant and its potential applications in various fields, including medicinal and pharmaceutical research.

Table 1: GC-MS analysis of methanol extract of *A. adenophora* leaf, stem and root

S. No.	Compounds Name	%Area			Ret. Time	Mol. Formula	Ret. Index	Mol. Weight
		MEL	MES	MER				
1	Betulin	-	-	11.27	31.360	C ₃₀ H ₅₀ O ₂	-	466
2	Lup-20(29)-ene-3,28-diol	-	-	4.93	29.887	C ₃₀ H ₅₀ O ₂	-	442
3	Lupa-13(18),20(30)-dien-3-yl-acetate	-	-	12.23	27.892	C ₃₂ H ₅₀ O ₂	-	466
4	Gama-sitosterol	-	-	3.58	26.429	C ₂₉ H ₅₀ O	2731	414
5	Stigma sterol	4.75	-	5.28	25.759	C ₂₉ H ₄₈ O	2739	412
6	Kolavenol	4.95	-	-	18.078	C ₂₀ H ₃₄ O	-	290

Ethanol extract: GC-MS analysis of ethanol extracts of *A. adenophora* leaves, stem and root part were carried out. A total of 14 phytoconstituents were reported in leaves, 29 in stem and 43 in root in this analysis. As shown in the table 5.3.2, 10 major compounds were detected from the ethanol extract of *A. adenophora* leaves, stem and root. The maximum concentration was detected for Delta.9-Capnellene-2-Epsilon-ol-8-one(29.25 %) for leaf while for stem was 24(S) Ethyl-3 Alpha,5 Alpha-cyclocholest-22(E)-en-6-one(15.96%) and in root for Lupa-13(18),20(30)-Dien-3-yl-acetate(12.23 %). Molecular formula with molecular weight of all the major compounds of ethanol extracts of leaf, stem and root are given in comparative manner of percentage in the table

Aqueous extract: GC-MS analysis of aqueous extract of *A. adenophora* leaves, stem and root part were carried out. A total of 19 phytoconstituents were reported in leaves, 30 in stem and 26 constituents in root extracts in this analysis.

Antioxidant activity of *A. adenophora* leaves: It seems like you are about to provide information about the antioxidant activity of different solvent extracts of *A. adenophora* leaves. However, it appears that you haven't yet provided the specific results or outcomes of the study. If you have data or results from the antioxidant activity assays and would like to share them or have any specific questions related to the study, please provide the details

Table 2: Radical scavenging activity of leaves extracts of *A. adenophora* at different concentrations

Antioxidant activity(%inhibition)					
Concentration(µg/ml)	Methanol	Ethanol	Hexane	Aqueous	Ascorbic acid
10	61.27±0.20	59.08± 0.23	5.57±0.36	50.11±0.73	82.49±0.76
30	64.89±0.20	62.89±0.23	9.11±0.33	52.53±0.26	84.82±0.52
50	68.47±0.26	66.74±0.20	12.77±0.23	55.26± 0.07	87.55±0.40
70	71.74±0.23	69.81± 0.27	16.66±0.40	59.11±0.19	91.74±0.69
90	75.51±0.17	73.28±0.23	19.70±0.23	62.04±0.23	92.07±0.58
100	77.24±0.26	75.59±0.26	21.28±0.16	63.62±0.20	94.34±0.40

Table 3: Ferric reducing antioxidant power of leaves extracts of *A.adenophora* at different concentration

Antioxidant activity(%inhibition)					
Concentration($\mu\text{g/ml}$)	Methanol	Ethanol	Hexane	Aqueous	Ascorbic acid
10	65.38 \pm 0.19	58.30 \pm 0.21	48.69 \pm 0.17	17.31 \pm 0.21	73.64 \pm 0.11
30	68.79 \pm 0.23	61.19 \pm 0.20	51.14 \pm 0.20	20.38 \pm 0.33	76.66 \pm 0.16
50	71.21 \pm 0.11	63.80 \pm 0.18	52.59 \pm 0.17	22.69 \pm 0.20	78.20 \pm 0.15
70	74.10 \pm 0.20	67.04 \pm 0.12	55.16 \pm 0.17	25.11 \pm 0.12	79.77 \pm 0.09
90	76.91 \pm 0.25	70.10 \pm 0.22	57.59 \pm 0.15	27.65 \pm 0.25	82.91 \pm 0.10
100	78.36 \pm 0.23	72.05 \pm 0.15	59.69 \pm 0.15	28.71 \pm 0.15	84.76 \pm 0.16

In this investigation the highest ferric Reducing antioxidant power was observed for methanol extract with an IC₅₀ value of 7.59 $\mu\text{g/ml}$, followed by ethanol extract 7.11 $\mu\text{g/ml}$ and hexane extract 29.32 $\mu\text{g/ml}$ but still weaker than ascorbic acid. The aqueous extract showed lowest ferric reducing antioxidant power within 50 value 174.12 $\mu\text{g/ml}$ respectively.

Table 4: Metal-Chelating activity of leaf extracts of *A. adenophora* at different concentration

Antioxidant activity(%inhibition)					
Concentration($\mu\text{g/ml}$)	Methanol	Ethanol	Hexane	Aqueous	EDTA
10	20.64 \pm 0.20	7.58 \pm 0.32	37.28 \pm 0.27	13.97 \pm 0.37	66.95 \pm 0.24
30	24.79 \pm 0.25	9.96 \pm 0.20	41.95 \pm 0.28	17.78 \pm 0.20	68.75 \pm 0.25
50	28.27 \pm 0.45	14.81 \pm 0.28	46.82 \pm 0.28	21.12 \pm 0.20	70.43 \pm 0.35
70	32.23 \pm 0.20	18.44 \pm 0.29	52.07 \pm 0.29	24.50 \pm 0.25	71.96 \pm 0.18
90	37.66 \pm 0.25	21.26 \pm 0.39	54.97 \pm 0.20	27.41 \pm 0.20	73.31 \pm 0.36
100	41.10 \pm 0.29	22.64 \pm 0.20	56.45 \pm 0.25	29.99 \pm 0.20	76.51 \pm 0.28

Antioxidant activity of *A.adenophora* stem: Antioxidant activity of different solvent extracts of *A. adenophora* stem was examined by following three standard assays. The outcomes of the present study areas under.

Table 5: Radical scavenging activity of stem extracts of *A. adenophora* at different concentration

Antioxidant activity(%inhibition)					
Concentration($\mu\text{g/ml}$)	Methanol	Ethanol	Hexane	Aqueous	Ascorbic acid
10	67.40 \pm 0.10	59.26 \pm 0.17	12.39 \pm 0.20	69.09 \pm 0.23	82.49 \pm 0.76
30	72.46 \pm 0.38	60.95 \pm 0.14	15.66 \pm 0.20	72.53 \pm 0.21	84.82 \pm 0.52
50	75.51 \pm 0.17	63.52 \pm 0.11	18.70 \pm 0.29	75.74 \pm 0.32	87.55 \pm 0.40
70	78.21 \pm 0.17	66.70 \pm 0.20	21.81 \pm 0.20	78.65 \pm 0.46	91.74 \pm 0.69
90	81.32 \pm 0.14	69.80 \pm 0.17	25.52 \pm 0.17	81.18 \pm 0.17	92.07 \pm 0.58
100	84.22 \pm 0.20	72.20 \pm 0.20	26.91 \pm 0.17	82.87 \pm 0.17	94.34 \pm 0.40

Ferric reducing antioxidant power (FRAP) : The FRAP (Ferric Reducing Antioxidant Power) assay is indeed considered a speedy and sensitive method for assessing the antioxidant potential of compounds or extracts. In this assay, a blue-colored complex (ferrous-TPTZ complex) is formed as a result of the reduction of ferric iron in the FRAP reagent, and the color change is used to quantify the reducing power of antioxidants in the test samples. A higher FRAP value indicates a greater antioxidant potential

Table 6: Radical scavenging, ferric reducing and metal chelating activities of stem extracts of *A.adenophora*

Plant extracts	IC ₅₀ value($\mu\text{g/ml}$)		MC Aassay
	DPPH assay	FRAP assay	
Methanol	7.34 \pm 0.05	9.36 \pm 0.03	9.58 \pm 0.06
Ethanol	8.43 \pm 0.02	7.02 \pm 0.02	9.39 \pm 0.08
Aqueous	7.23 \pm 0.02	124.01 \pm 0.5	86.94 \pm 0.4
Hexane	185.82 \pm 1.2	131.51 \pm 0.5	120.58 \pm 0.7
Ascorbic acid /EDTA	6.05 \pm 0.05	6.78 \pm 0.01	7.46 \pm 0.04

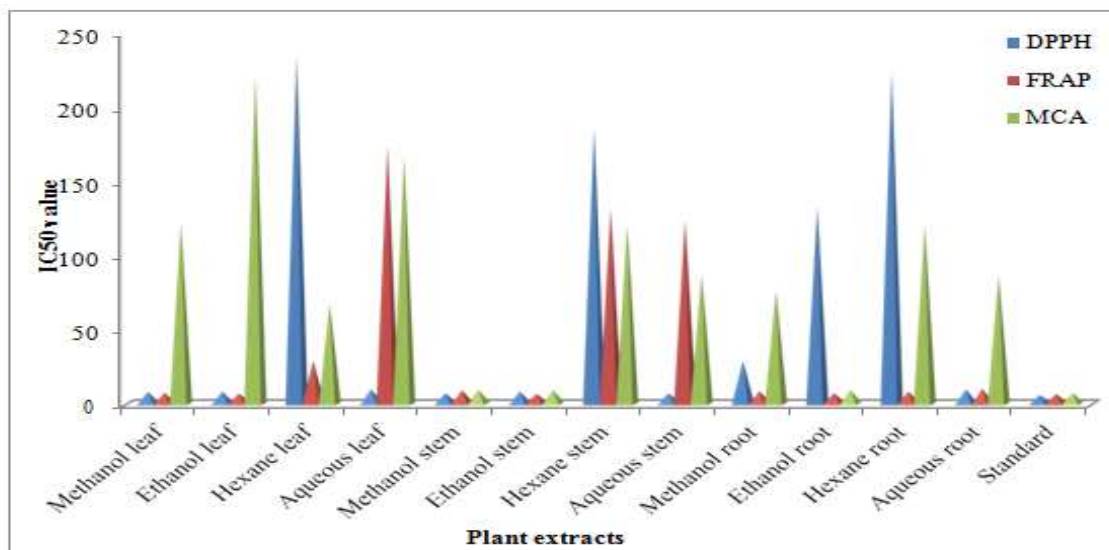


Fig. 1. Antioxidant Potential (in the form of IC50 value) of different solvent extracts of A. adenophora (leaf, stem and root), standard-Ascorbic acid for DPPH, FRAP and EDTA for MCA.

Sonchus wightianus DC. : Analyzing the antimicrobial and antioxidant potential of different parts of a plant like *S. wightianus* (scientifically known as *Strobanthes wightianus*) using various solvent extracts is a common approach in botanical and pharmaceutical research. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful technique for identifying and quantifying the chemical compounds present in these extracts. This approach can help in understanding the bioactive compounds responsible for these properties

Table: 7 Radical scavenging activity of leaves extracts of S. wightianus at different concentration

Antioxidant activity(%inhibition)					
Concentration (µg/ml)	Methanol	Ethanol	Hexane	Aqueous	Ascorbic acid
10	48.06±0.11	44.33±0.20	12.33±0.17	49.30±0.14	82.49±0.76
30	49.67±0.26	46.29±0.20	14.24±0.15	51.46±0.25	84.82±0.52
50	52.41±0.26	47.96±0.17	16.57±0.20	52.68±0.14	87.55±0.40
70	53.92±0.14	49.27±0.26	19.45±0.20	54.69±0.14	91.74±0.69
90	56.90±0.14	51.31±0.14	22.69±0.20	56.53±0.12	92.07±0.58
100	58.63 ±0.14	53.18±0.29	24.87±0.26	57.73±0.17	94.34±0.40

Antioxidant activity of S.wightianus root: Certainly, I'm here to help discuss the outcomes of the study on the antioxidant activity of different solvent extracts of *S. wightianus* root using three standard assays.

Antioxidant activity of A.nilagirica leaves: Antioxidant activity of different solvent extracts of *A. nilagirica* leaves was examined by following three standard assays. The outcomes of the present study areas under.

Table: 8 Radical scavenging activity of leaves extracts of A. nilagirica at different concentration

Antioxidant activity(%inhibition)					
Concentration (µg/ml)	Methanol	Ethanol	Hexane	Aqueous	Ascorbic acid
10	56.81±0.20	53.06±0.21	2.20±0.20	51.16±0.41	82.49±0.76
30	59.07±0.20	55.43±0.17	5.41±0.17	54.68±0.20	84.82±0.52
50	61.40±0.17	58.00±0.20	7.62±0.24	57.92±0.17	87.55±0.40
70	62.94±0.17	60.17±0.20	10.23±0.17	60.77±0.17	91.74±0.69
90	64.76±0.13	62.94±0.17	13.24±0.27	64.80±0.17	92.07±0.58
100	67.17 ±0.17	65.12±0.20	15.17±0.18	66.22±0.17	94.34±0.40

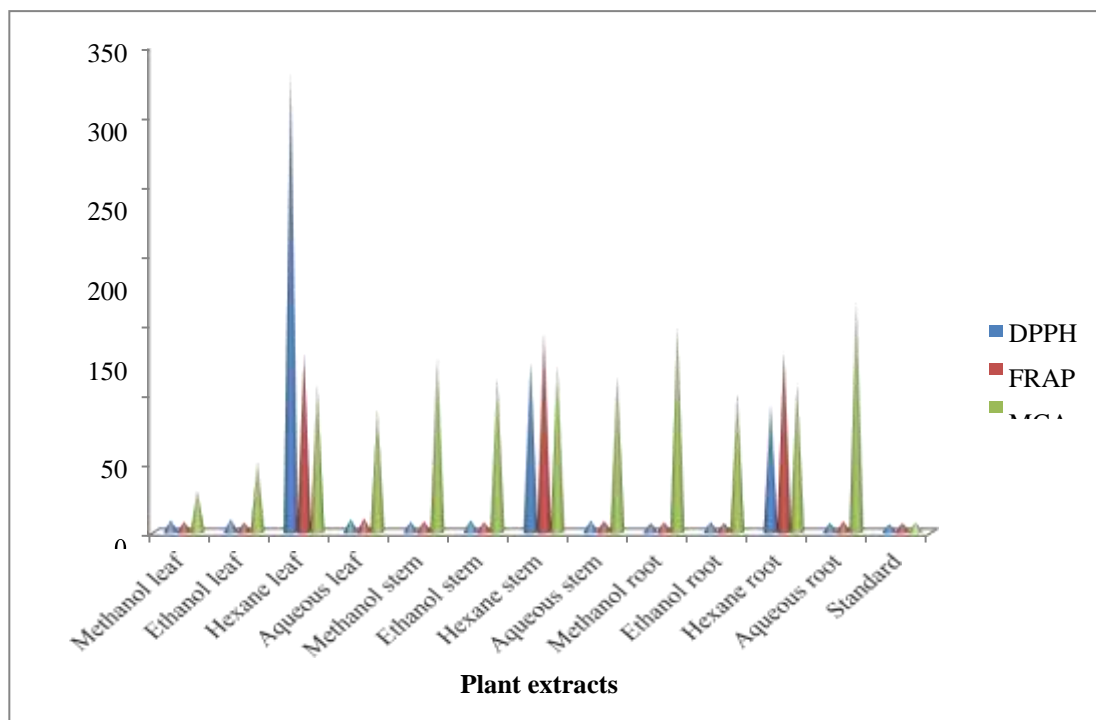
Metal chelating antioxidant power (MCA) : The chelating effect of different solvent extracts of *A. nilagirica* root on metal ions using the Metal-Chelating Antioxidant (MCA) assay, the following findings were reported Order of Metal-Chelating Antioxidant Activity: The ability of the test samples to chelate metal ions was observed in the following order, from highest to lowest:

- Ethanol extract: IC50 value of 99.90 µg/ml (strongest metal-chelating activity)

- Hexane extract: IC50 value of 107.72 $\mu\text{g/ml}$
- Methanol extract: IC50 value of 147.57 $\mu\text{g/ml}$
- Aqueous extract: IC50 value of 165.98 $\mu\text{g/ml}$ (weakest metal-chelating activity)

Table: 9 Metal-Chelating antioxidant power of root extracts of *A. nilagirica* at different concentration

Antioxidant activity(%inhibition)					
Concentration($\mu\text{g/ml}$)	Methanol	Ethanol	Hexane	Aqueous	EDTA
10	7.79 \pm 0.23	37.55 \pm 0.17	23.16 \pm 0.28	5.52 \pm 0.33	66.95 \pm 0.24
30	12.94 \pm 0.32	40.40 \pm 0.11	27.80 \pm 0.20	12.95 \pm 0.29	68.75 \pm 0.25
50	20.09 \pm 0.24	43.68 \pm 0.14	32.49 \pm 0.20	18.56 \pm 0.20	70.43 \pm 0.35
70	27.08 \pm 0.22	46.32 \pm 0.20	37.74 \pm 0.29	22.65 \pm 0.32	71.96 \pm 0.18
90	29.98 \pm 0.28	48.69 \pm 0.09	42.66 \pm 0.26	27.75 \pm 0.24	73.31 \pm 0.36
100	33.88 \pm 0.28	50.35 \pm 0.17	46.42 \pm 0.28	30.12 \pm 0.24	76.51 \pm 0.28

**Fig: Antioxidant potential (in the form of IC50 value) of different solvent extracts of *A. nilagirica* (leaf, stem and root), standard- Ascorbic acid for DPPH, FRAP and EDTA for MCA.**

Phytochemicals and Antioxidant Activity: Free radicals are unstable molecules that play a role in various health problems, and antioxidants help combat them. Antioxidants protect our bodies from degenerative diseases. Free radicals, often called reactive oxygen species (ROS), are generated during normal metabolic processes in our cells. These can be both beneficial and harmful to our bodies. The phytochemical analysis of different parts (leaves, stems, and roots) and various extracts (methanol, ethanol, and aqueous) of *A. adenophora*, *S. wightianus*, and *A. nilagirica* revealed the presence of various compounds such as phytol, stigmasterol, leden, n-hexadecanoic acid, isopropyl linoleate, squalene, ricinoleic acid, and its esters, palmitic acid. These compounds are responsible for the antioxidant properties of these plants. In this study, the methanol, ethanol, and aqueous extracts of *Ageratina adenophora* exhibited significant antioxidant activity. This activity is linked to the presence of certain compounds, such as phytol, stigmasterol, leden, 1,3,4,5-tetrahydroxy-cyclohexane carboxylic acid, and n-hexadecanoic acid, which are known for their antioxidant properties. The DPPH assay revealed that the methanol, ethanol, and aqueous extracts of *A. adenophora* leaf had significant antiradical scavenging potential. This is because these extracts act as hydrogen donors and singlet oxygen quenchers. The presence of phytol in the methanol extract was particularly associated with its high antiradical activity. Similarly, the methanol and ethanol extracts of the *A. adenophora* stem also showed significant antiradical activity in the DPPH assay. The high ferric-reducing antioxidant potential of these extracts suggests the presence of compounds that can donate electrons and transform free radicals into more stable products. The presence of stigmasterol in the methanol extract of the stem is related to its antioxidant activity.

In simpler terms, the extracts from *A. adenophora* contain compounds that can help neutralize harmful free radicals, which are associated with various health issues. These compounds act as antioxidants, and the extracts from both the leaves and stems of *A. adenophora* have shown significant antioxidant properties in the tests conducted.

5. ACKNOWLEDGEMENT:

I would like to show my sincere gratitude and respect to my mentor Dr. Vivek Gupta Professor, Monad University ,N.H. 9, Delhi Hapur Road Village & Post Kastla, Kasmabad, Pilkhuwa, Uttar Pradesh for providing me with the necessary guidance and helping me throughout my work

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