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Screening of phytochemical and antioxidant properties of *moringa oleifera* and *Andrographis paniculata*

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ABSTRACT

This study examined the phytochemical and antioxidant potential of extracts from two valuable medicinal plants-*Andrographis paniculata* and *Moringa oleifera*. Phytochemical analysis of both the extracts revealed that they are rich in various compounds such as Flavonoids, Tannins and Phenols. Screening of antioxidant property confirmed a correlation between their phytochemical richness and the ability to scavenge free radicals. All extracts showed a dose dependent ability to inhibit free radicals confirming their antioxidant nature. Petroleum ether extract of *Andrographis paniculata* displayed better activity, achieving a maximum inhibition rate of 48.11%, while aqueous *Moringa oleifera* extracts displayed maximum inhibition at 31.74%. This confirms a concentrated phytochemical profile in *Andrographis paniculata* and hence higher antioxidant potential. The current results suggest that both plants are promising sources of natural antioxidants with *Andrographis paniculata* having particularly strong potential for therapeutic and health-related uses**.**

Keywords: - *Andrographis paniculata*, *Moringa oleifera*, Phytochemicals, Antioxidants.

INTRODUCTION

A biological condition known as oxidative stress is brought on by an overabundance of reactive oxygen species (ROS) relative to the body's antioxidant capacity (Liguori *et al.,* 2018). Lipids, proteins, and DNA are among the vital cellular constituents that suffer oxidative damage as a result of this imbalance. Aging and the emergence of chronic illnesses like diabetes, cancer, heart disease, and neurological disorders are linked to this kind of damage (Liguori *et al.,* 2018; Kaur & Arora, 2022). Antioxidants that neutralize free radicals, chelate metal ions, and boost native antioxidant enzymes help reduce oxidative stress, which is a major therapeutic objective (El-Beltagi *et al.,* 2023). Natural antioxidants made from plants are becoming more considered due to risk about the safety and possible toxicity of synthetic antioxidants. Beneficial phytochemicals found in these medicinal plants, such as polyphenols, flavonoids, and terpenoids, have strong antibacterial, anti-inflammatory, and antioxidant properties. *Andrographis paniculata* and *Moringa oleifera* are two notable examples. The anti-inflammatory, antimicrobial, and neuroprotective properties of *Moringa oleifera* are attributed to its high concentration of vitamins A, C, and E as well as phytochemicals like flavonoids and phenolic acids (Ullah *et al.,* 2022; Herman Lara *et al.,* 2024). More than forty compounds are found in its leaves, including potent antioxidants like quercetin, kaempferol, and gallic acid (Makita *et al.,* 2023; Thitivichienler *et al.,* 2024). Similarly, the primary active ingredients of *Andrographis paniculata,* which has long been used for its hepatoprotective and immunomodulatory properties, are flavonoids like luteolin and apigenin and diterpenoids like andrographolide (Lim *et al.,* 2021). This plant's extracts have been shown to have protective effects, such as reducing reactive oxygen species (ROS) in UV-exposed skin cells (Liu *et al.,* 2024), demonstrating strong cytotoxic and antioxidant effects on leukemia cells, and enhancing the heart's antioxidant defenses against oxidative stress in animal models (Gnanadeepan *et al.,* 2024; Aziz *et al.,* 2025).

Despite the numerous individual studies on these plants, a comparative assessment of their antioxidant capacities using uniform analytical protocols is lacking (Choudhary *et al.,* 2023). Establishing such data is essential for evaluating their relative effectiveness as natural antioxidants and for guiding their use in

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pharmaceutical and nutraceutical formulations. This study addresses this gap by conducting a preliminary phytochemical screening and antioxidant evaluation of leaf extracts from *Moringa oleifera* and *Andrographis paniculata***.** The assay employed is a hydrogen peroxide scavenging assay, which provides a preliminary assessment of the extracts antioxidant potential. This study provides a comprehensive analysis of the phytochemical composition and antioxidant activity of *Moringa oleifera* and *Andrographis paniculata* leaf extracts. The findings are expected to validate traditional medicinal uses inform the development of antioxidant-rich functional foods and herbal therapeutics and serve as a foundation for further mechanistic and clinical studies.

MATERIALS AND METHODS

Plant Identification

Fresh, healthy leaves of *Andrographis paniculata* and *Moringa oleifera* were collected from their natural habitats in Chhattisgarh, India. The State Forest Research Institute (SFRI), Jabalpur, identified and verified the plant specimens. Phytochemicals based on polarity were extracted using analytical grade solvents in compliance with standard phytochemical extraction protocols (Azwanida, 2015).

Processing of Plant Material

To remove dust and other surface impurities, freshly collected *Andrographis paniculata* and *Moringa oleifera* leaves were first rinsed with tap water and then rinsed again with distilled water (Kosalec *et al.,* 2009). To eliminate moisture and protect heat-sensitive phytochemicals, the cleaned leaves were subsequently shade-dried for roughly 15 days at room temperature (25–30°C). A sterile mechanical grinder was used to grind the leaves finely once they had completely dried. To preserve the integrity of the bioactive ingredients until further use, the resultant powder was kept in airtight containers in a cool, dry and dark location (Pandey & Tripathi, 2014).

Preparation of Plant Extracts

A Soxhlet apparatus was used to extract solvents successively from about 25 grams of dried and powdered *Andrographis paniculata* and *Moringa oleifera* leaves. Four analytical-grade solvents Distilled water, Methanol, Chloroform, and Petroleum ether were used in sequential extraction, each applied in ascending order of polarity. 250 ml of each solvent were used and extraction was done at the boiling point of each solvent. Until the siphon solution became clear, signifying the depletion of extractable phytochemicals, the procedure was carried out repeatedly (Tiwari *et al.,* 2011).

Preliminary tests of Plant extracts

a) Qualitative Test for Flavonoids (Alkaline Reagent Test)

2-3 drops of 2% sodium hydroxide solution were combined with 2 ml of each plant extract. The presence of Flavonoids was indicated by the formation of an intense yellow color. The test was confirmed when a few drops of diluted Hydrochloric acid (HCl) were added later, causing the yellow color to vanish (Sharma *et al.,* 2020; Pratap *et al.,* 2022).

b) Qualitative Test for Tannin (Ferric chloride test)

2 ml of plant extract was combined with 2–3 drops of 1% Ferric Chloride ($FeCl_3$) solution. The formation of brownish-green or blue-black coloration indicated the presence of tannins (Patel *et al.,* 2021; Rahman *et al.,* 2023).

c) Qualitative Test for Terpenoids (Salkowski Test)

2 ml of Chloroform was mixed with 0.5 ml of the plant extract. To create a clear layer, 1 ml of concentrated Sulfuric acid was then carefully added to the mixture. A positive result for Terpenoids was indicated by the appearance of a reddish-brown color at the interface (Nisha *et al.,* 2021; Ezeonu*et al.,* 2022).

d) Qualitative Test for Cardiac Glycosides (Keller-Killiani Test)

2 ml of Glacial acetic acid with a few drops of a 5% Ferric Chloride solution were mixed with one ml of the plant extract. To form a clear layer, 1 ml of concentrated Sulfuric acid (H_2SO_4) was then carefully added along the test tube's side. Deoxysugars, which are indicative of cardiac glycosides, were present at the

interface as a brown ring (Khandelwal, 2008; Trease & Evans, 2009).

e) Qualitative Test for Phenolic Compounds (Ferric Chloride Test)

1 ml of plant extract was combined with 1 ml of 5% ferric chloride ($FeCl_3$) solution. The appearance of a blue-black coloration or precipitate indicated the presence of Phenolic groups in the extract (Kokate, 2005).

f) Qualitative Test for Steroids (Liebermann–Burchard Reaction)

2 ml of chloroform were mixed with one ml of plant extract. Then, to form a distinct layer, 2 ml of concentrated Sulphuric acid was cautiously added along the test tube's side. A positive result for steroidal compounds was indicated by the bottom layer turning red (Trease & Evans, 2009).

g) Qualitative Test for Alkaloids (Mayer's Test)

Few drops of Mayer's reagent (Potassium Mercuric Iiodide solution) were added to 2 ml of extract. A positive alkaloid result was indicated by the formation of a creamy white precipitate (Parekh & Chanda, 2007; Ayoola *et al.,* 2008).

h) Qualitative Test for Saponins (Foam Test)

5 ml of distilled water was added to a test tube containing 2 ml of plant extract. The mixture was vigorously shaken for 30 seconds. A positive saponin result was demonstrated by development of stable, persistent foam that persisted for at least ten minutes (Obadoni & Ochuko, 2001).

Evaluation of Antioxidant Activity

The hydrogen peroxide (H_2O_2) scavenging assay was used to evaluate the antioxidant activity of leaf extracts from *Moringa oleifera* and *Andrographis paniculata* (Muhammad *et al.,* 2022; Syahputra *et al.,* 2023).Using an appropriate solvent, various concentrations of each plant extract (2, 4, 6, 8, and 10 µl) were made in a final volume of 1 ml. A common antioxidant control was ascorbic acid. 0.6 ml of hydrogen peroxide solution (50 mm in phosphate buffer, pH 7.4) was added to each sample. For 5 minutes, the reaction mixtures were incubated at room temperature. After incubation, the absorbance of each sample was measured at 230 nm using a UV–Visible spectrophotometer (Adeoye *et al.,* 2022). The percentage scavenging activity was calculated using the following formula:

$$\% \text{ Scavenging Activity} = (1 - Ac/As) \times 100$$

Where:

A_s = Absorbance of the sample (extract + H_2O_2)
A_c = Absorbance of the control (H_2O_2 alone)

RESULTS

Phytochemical analysis of plant extracts

***Andrographis paniculata*:** Several bioactive compounds were found in the extracts of *Andrographis paniculata* after phytochemical screening (Table 1). All eight Phytochemicals, including Flavonoids, Tannins and Alkaloids, were detected in the aqueous and methanol extracts. However, Only Terpenoids and Glycosides were found in the petroleum ether extract, whereas a greater range of these compounds were also found in the chloroform extract.

Table 1: Preliminary phytochemical analysis- *Andrographis paniculata*

S. No.	Phytochemicals	Aqueous	Methanol	Chloroform	Petroleum ether
1.	Flavonoids	Positive	Positive	Negative	Negative
2.	Tanins	Positive	Positive	Positive	Positive
3.	Terpenoids	Positive	Positive	Positive	Positive
4.	Glycosides	Positive	Positive	Positive	Positive
5.	Phenols	Positive	Positive	Positive	Negative
6.	Steroids	Positive	Positive	Positive	Negative
7.	Alkaloids	Positive	Positive	Positive	Negative
8.	Saponins	Positive	Positive	Positive	Negative

***Moringa oleifera*:** The phytochemical distribution in *Moringa oleifera* extracts varied according to the solvent used (Table 2). Numerous substances, such as Phenols, Terpenoids, and Tannins, were present in the methanol extract. Flavonoids, Tannins and Alkaloids were present in the chloroform extract. The profiles of the petroleum ether and aqueous extracts, on the other hand, were more selective and showed favourable outcomes for fewer compounds.

Table 2: Preliminary phytochemical analysis- *Moringa oleifera*

S. No.	Phytochemicals	Aqueous	Methanol	Chloroform	Petroleum ether
1.	Flavonoids	Negative	Positive	Positive	Negative
2.	Tanins	Positive	Positive	Positive	Negative
3.	Terpenoids	Positive	Positive	Positive	Negative
4.	Glycosides	Negative	Positive	Negative	Positive
5.	Phenols	Positive	Positive	Positive	Negative
6.	Steroids	Negative	Negative	Positive	Positive
7.	Alkaloids	Negative	Negative	Positive	Negative
8.	Saponins	Positive	Positive	Negative	Positive

Evaluation of Antioxidant Activity

The absorbance values obtained for different concentrations of ascorbic acid along with the calculated average absorbance and inhibition percentages are presented in Table 3 and Figure 1. The blank (control) absorbance was determined to be 2.864.Calculated IC50: 8.324 µg/ml (Concentration at 50% Inhibition).

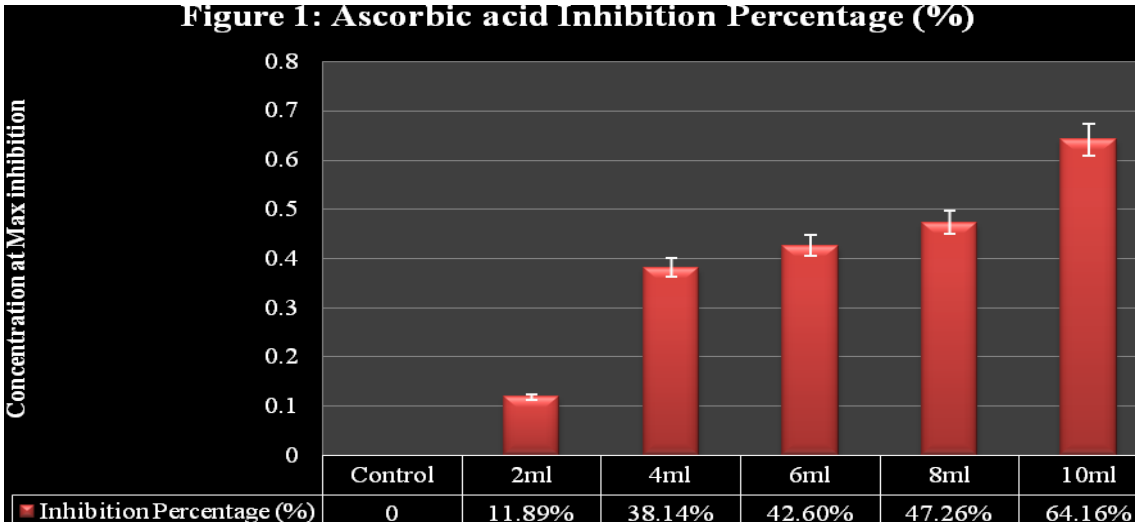
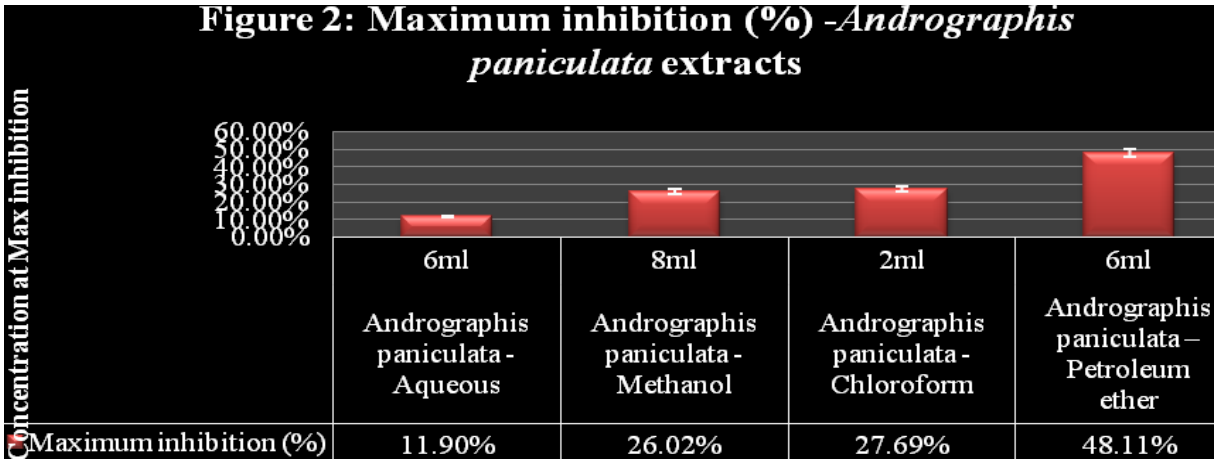


Table 3: Absorbance and hydrogen peroxide scavenging activity of ascorbic acid

S. No.	Concentration (µg/ml)	1st Absorption	2nd Absorption	3rd Absorption	Average absorbance	Inhibition Percentage (%)
1.	2ml	2.530	2.500	2.540	2.864	11.89%
2.	4ml	1.851	1.717	1.747	2.523	38.14%
3.	6ml	1.664	1.548	1.720	1.772	42.60%
4.	8ml	1.554	1.435	1.542	1.644	47.26%
5.	10ml	1.022	1.033	1.024	1.026	64.16%

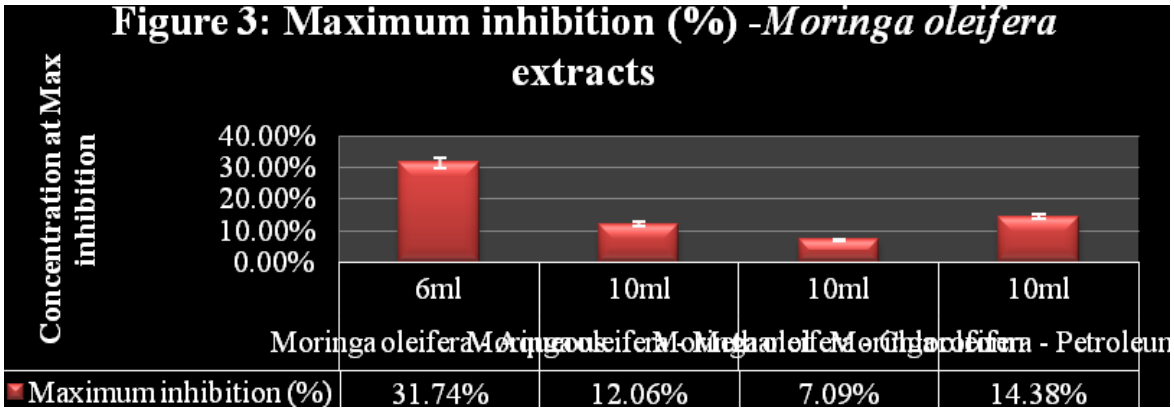
The in vitro evaluation of eight plant extracts; four from *Andrographis paniculata* and four from *Moringa oleifera*, successfully demonstrated their capacity to inhibit free radicals (Table 4). All extracts exhibited measurable antioxidant properties within the tested concentration range of 2-10 µg/ml. The most prominent findings were observed in Petroleum ether extract of *Andrographis paniculata* achieved a substantial maximum



inhibition percentage of 48.11% (Figure 2). This result is highly encouraging as it approaches the 50% threshold, indicating significant antioxidant potential. Similarly, aqueous extract of *Moringa oleifera* showed commendable maximum inhibition of 31.74 %, (Figure 3) confirming its free radical scavenging capabilities. The data clearly indicates a dose-dependent response, providing a robust basis for the next phase of the study.

Table 4: Maximum inhibition (%) - *Andrographis paniculata* and *Moringa oleifera* extracts

Extract source	Maximum inhibition (%)	Concentration at Max inhibition
Andrographis paniculata -Aqueous	11.90%	6ml
Andrographis paniculata -Methanol	26.02%	8ml
Andrographis paniculata -Chloroform	27.69%	2ml
Andrographis paniculata – Petroleum ether	48.11%	6ml
Moringa oleifera - Aqueous	31.74%	6ml
Moringa oleifera - Methanol	12.06%	10ml
Moringa oleifera - Chloroform	7.09%	10ml
Moringa oleifera - Petroleum ether	14.38%	10ml



CONCLUSION

The Phytochemical analysis revealed that both *Andrographis paniculata* and *Moringa oleifera* extracts are rich in various compounds, including Flavonoids, Tannins and Phenols. These findings correlate directly with their antioxidant activity. All tested extracts demonstrated a dose-dependent capacity to inhibit free radicals. Petroleum ether extract of *Andrographis paniculata* extract showed the highest activity with a maximum inhibition of 48.11%, while an aqueous extract of *Moringa oleifera* achieved 31.74% inhibition. The superior antioxidant potential of *Andrographis paniculata* is likely due to its richer phytochemical profile. These results confirm that both plants are promising sources of natural antioxidants.

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