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Antioxidant activity of a Polyherbal formulation 'ACHF-01' by DPPH radical scavenging assay

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Abstract

This study evaluates the antioxidant potential of an herbal formulation through the 2, 2-dipheny l – 1- picrylhydrazyl (DPPH) free radical scavenging assay. Polyherbal formulation 'ACHF-01' was tested, with ascorbic acid serving as the positive control. The percentage inhibition of DPPH radicals of sample 'ACHF-01' demonstrating the 55.78% activity. These findings highlight the potential of specific herbal combinations as natural antioxidants and suggest the need for further phytochemical analysis and *In vivo* validation.

Keywords: Antioxidant; DPPH assay; Herbal formulation; Free radical scavenging; Phytochemical

1. Introduction

Oxidative stress, resulting from an imbalance between the generation of reactive oxygen species (ROS) and the body's ability to neutralize them using antioxidants. Antioxidants play a pivotal role in the pathophysiology of numerous chronic diseases, including cancer, cardiovascular diseases, diabetes, neurodegenerative disorders, and aging [1, 2]. ROS such as superoxide anion ($O_2\bullet^-$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2) are highly reactive molecules that can damage biomolecules like DNA, proteins and lipids, leading to cell dysfunction and death [3]. To counteract the effects of oxidative stress, both endogenous (e.g., catalase, superoxide dismutase) and exogenous antioxidants (e.g., polyphenols, flavonoids, vitamins C and E) are essential. Herbal formulations, which combine multiple plants, are known to provide synergistic therapeutic effects, including enhanced antioxidant activity due to the presence of diverse phytochemicals like flavonoids, tannins, alkaloids, and phenolic acids [4].

The evaluation of antioxidant potential *In vitro* is commonly performed using the 2, 2-dipheny l – 1- picrylhydrazyl (DPPH) assay. This method is based on the ability of antioxidants to donate hydrogen atoms to the DPPH radical, a stable nitrogen-centered free radical that exhibits a deep violet colour. Upon reduction, DPPH becomes colourless or pale yellow, and the degree of discoloration correlates with the scavenging ability of the tested compound [5, 6]. The assay is simple, reproducible and requires only basic instrumentation, making it an ideal screening tool for antioxidant activity in herbal samples.

This research contributes to the development of plant-based therapeutic agents with antioxidant benefits which can be used in many diseases like Cancer, COVID- 19, Dengue, Chikungunya, etc.

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2. Material and methods

2.1. Chemicals and Reagents

The chemicals and reagents used in this study were 2, 2- Diphenyl – 1- picrylhydrazyl (DPPH), Ascorbic acid (standard antioxidant), Methanol, Distilled water. DPPH was selected for its stable free radical properties and its sensitivity to hydrogen or electron-donating antioxidant compounds [6]. Ascorbic acid was used as the positive control due to its well-established antioxidant potential [7].

2.2. Herbal Formulation and Sample Preparation:

The study involved Polyherbal formulation, labelled 'ACHF-01' was composed of varying combination (given in table 1) of ethanolic extract, which was prepared using Soxhlet's extraction method [8, 9] from dried and powdered plant materials.

The filtrates were concentrated under reduced pressure using a rotary evaporator and stored in airtight containers at 4°C until further use. For the antioxidant assay, a stock solution of sample was prepared at a concentration of 1 mg/mL in methanol and serially diluted as required.

Table 1 Composition of Polyherbal Formulation 'ACHF-01' with medicinal properties

Sr. No.	Scientific Names of Plants	Family	Medicinal Properties	References
1.	*Justicia adhatoda* L.	*Acanthaceae*	Antitussive, expectorant, anti-inflammatory and bronchodilator	[10]
2.	*Pulicaria wightiana* (DC.) C. B. Clarke	*Asteraceae*	Anti-inflammatory and contains bioactive flavonoids and sesquiterpene lactones	[11]
3.	*Azadirachta indica* A. Juss.	*Meliaceae*	Antiviral, hepatoprotective and antioxidant	[12]
4.	*Glycyrrhiza glabra* L.	*Fabaceae*	Antioxidant, anti-inflammatory, hepatoprotective and antiviral	[13]
5.	*Piper nigrum* L.	*Piperaceae*	Antioxidant, antibacterial, anti-inflammatory, immunomodulatory, etc	[14]

2.3. Apparatus and Equipment

Eppendorf tubes (1.5 mL and 2 mL), Glass vials (5 mL and 30 mL), Micropipettes (20–200 µL and 100–1000 µL), and UV-Visible spectrophotometer were used. All equipment was cleaned and calibrated prior to the experiment. Spectrophotometric readings were recorded at 517 nm, the absorption maximum of DPPH radicals in methanol [5].

2.4. DPPH Radical Scavenging Assay

The antioxidant activity was assessed using the DPPH free radical scavenging assay as described by Blois (1958) and modified by other researchers [15, 16]. The procedure was as follows:

- A 0.1 mM DPPH solution was prepared in methanol and kept in the dark to protect it from light-induced degradation.
- In a clean 5 mL glass vial, 1 mL of DPPH solution was mixed with 1 mL of sample solution ('ACHF-01').
- The mixture was incubated at room temperature in the dark for 30 minutes.

The absorbance was measured at 517 nm against a methanol blank using a UV-Visible spectrophotometer. Ascorbic acid was tested under the same conditions as a reference standard.

2.5. Calculation of Antioxidant Activity

The antioxidant activity was expressed as the percentage of DPPH radical inhibition using the following formula:

$$\text{DPPH Scavenged (\%)} = (A - S) / A \times 100$$

Where,

- A = Absorbance of the blank (DPPH only)
- S = Absorbance of the sample

Each experiment was performed in triplicate, and the results were expressed as mean ± standard deviation.

3. Results

The antioxidant activity of the 'ACHF-01' was evaluated using the DPPH radical scavenging assay and compared with the standard antioxidant, ascorbic acid. The results are presented in Table 2.

Table 2 DPPH Radical Scavenging Activity of Test Samples

Sample	Absorbance (S)	DPPH Scavenged %
DPPH Control (A)	0.6443	—
Ascorbic Acid (Positive Control)	0.0250	96.12
Polyherbal Formulation 'ACHF-01'	0.2849	55.78

Figure 1 Image of final vials analysed under UV-Spectrophotometer

The 'ACHF-01' demonstrated a moderate free radical scavenging activity of 55.78%, indicating the presence of active phytochemicals with antioxidant potential. As expected, ascorbic acid exhibited a significantly higher activity of 96.12%, confirming its role as a potent antioxidant reference standard.

4. Discussion

The present study confirms the antioxidant potential of the polyherbal formulation 'ACHF-01' through the DPPH assay. Although the scavenging activity (55.78%) was lower than that of ascorbic acid (96.12%), the formulation still exhibited noteworthy free radical quenching capability. This suggests that the bioactive constituents present in the selected medicinal plants contribute synergistically to its antioxidant profile.

The promising antioxidant activity observed in 'ACHF-01' highlights the potential of combining medicinal plants to achieve effective free radical scavenging. The results suggest that optimising factors such as the proportion of ingredients, extraction method, and synergistic interactions among phytoconstituents could further enhance the formulation's efficacy. Moreover, the *In vitro* nature of the assay provides only preliminary insight. It is essential to validate the findings using *In vivo* models and assess parameters like oxidative stress biomarkers, bioavailability, and toxicity to determine therapeutic viability.

5. Conclusion

The study demonstrates that the polyherbal formulation 'ACHF-01' exhibits appreciable antioxidant activity, scavenging 55.78% of DPPH radicals *In vitro*. Although less potent than ascorbic acid, the formulation showed promise as a natural antioxidant source, likely due to the synergistic effects of its phytoconstituents.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declared no conflict of interest regarding the publication of this research article.

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