

In- Vitro Evaluation of DPPH Radical Scavenging Activity of Two Herbal Formulations

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ABSTRACT

The present study evaluates the antioxidant potential of two herbal formulations: ACHF-06, prepared from the fruits of *Stephanotis volubilis* and ACHF- 07, the traditional spice fruits *Cuminum cyminum* and *Trachyspermum ammi*. This study appraises the in- vitro antioxidant potential of both formulations through DPPH free radical scavenging assay. Five concentrations ranging from 20 – 100 µg/mL of two ethanolic extracts were tested in triplicate and the inhibition percentage, standard deviation and IC50 values were calculated. Both Herbal formulations demonstrated a concentration- dependent increase in radical scavenging activity. These findings highlight the potential of *S. volubilis*, *C. cyminum*, and *T. ammi* as natural antioxidant agents capable of ameliorating oxidative stress in optimized concentrations.

Keywords: Medicinal Plants, Antioxidant, DPPH assay, Herbal, Extract, *S. volubilis*, *C. cyminum*, *T. ammi*

I. INTRODUCTION

The human body and food systems both experience free radical responses. Reactive oxygen and nitrogen species are free radicals, which are essential to proper physiology. Due to oxidative stress caused by an imbalance in the body's antioxidant defence system and the creation of free radicals, an excess of these reactive species may be produced. Cellular damage and death may result from these ROS interactions with biomolecules. Antioxidant-rich fruits and

vegetables have been shown to provide protection against certain chronic illnesses. Antioxidants included in food can strengthen cellular defences and shield cellular components from oxidative damage [1; 2]. The preventive effects of antioxidants against oxidative stress-mediated disease processes in the body, have attracted increasing attention [3].

This study investigates antioxidant potential of two herbal formulations: ACHF-06 prepared from, the fruits of *Stephanotis volubilis* (L. f.) S. Reuss, Liede & Meve synonym *Dregea volubilis* (L. f.) Benth. ex Hook.

f. [4] contain phenolics and flavonoids known for strong free radical scavenging activity [5], traditionally used for its anti-inflammatory and antioxidant properties [6; 7]. The other formulation ACHF-07, ingredients include fruits of *Cuminum cyminum* L. and *Trachyspermum ammi* (L.) Sprague, both of which are aromatic culinary spices with well-documented antioxidant, antimicrobial and digestive benefits [8; 9]. *Cuminum cyminum* contains phenolic acids, flavonoids, and terpenes known for strong free radical-scavenging abilities [10], while *Trachyspermum ammi* is rich in thymol, p-cymene, and γ -terpinene, compounds associated with potent antioxidant and antimicrobial activities [11]. The 2, 2 - diphenyl - 1 - picrylhydrazyl (DPPH) method is one of the most widely accepted *in-vitro* assays for assessing free radical-scavenging activity due to its simplicity, reliability, and reproducibility [12]. Measuring the percentage inhibition of DPPH radicals allows for the determination of IC_{50} , which reflects the concentration required to scavenge 50% of free radicals and serves as a key indicator of antioxidant efficacy. This study aims to assess and compare the antioxidant activity of two formulations, ACHF-06 and ACHF-07 by analyzing their DPPH scavenging capacity and IC_{50} values. The research provides insight into their potential therapeutic applications in managing oxidative stress-related conditions.

II. METHODS AND MATERIAL

A. Plant Materials and Preparation of Extracts

Fresh fruits of *Stephanotis volubilis* were collected from Dr. Babasaheb Marathwada University Campus, Chhatrapati Sambhajanagar, M. S., India and fruits of *Cuminum cyminum* and *Trachyspermum ammi* from Dhabe Herbal Private Limited, Chhatrapati Sambhajanagar, M. S., India.

The plant materials were cleaned, shade-dried, and pulverized into fine powder. Each powdered sample was subjected to extraction using ethanol as a solvent

in a Soxhlet's apparatus [13; 14]. The ethanolic extracts were concentrated using a rotary evaporator under reduced pressure at 40–45°C and stored in airtight containers at 4°C.

B. Chemicals and Reagents

Ethanol, DPPH and Ascorbic acid as the standard antioxidant. All solutions were freshly prepared prior to analysis.

C. Preparation of Sample Solutions

Stock solutions (1 mg/ mL) of ACHF-06 and ACHF-07 were prepared by dissolving accurately weighed quantities of the dried extracts in Ethanol. Serial dilutions were prepared to obtain working concentrations of 20, 40, 60, 80 and 100 μ g/mL.

D. DPPH Radical Scavenging Assay

The *in-vitro* DPPH radical scavenging activity was assessed using, with minor adjustments, the method depicted by Blois, 1958 and Rosidah et al., 2008 [15; 16].

The DPPH Scavenging activity/ inhibition percentage (%) of two herbal formulations [17; 18] was calculated using the formula:

$$\text{Inhibition percentage (\%)} = \left(\frac{OD_{\text{control}} - OD_{\text{sample}}}{OD_{\text{control}}} \right) \times 100$$

Where, OD_{control} signifies the Optical density of the control = 1.006 (Absorbance of control group) and OD_{sample} specifies the Optical density of the test sample.

E. Procedure

In the 96 well plates, 10 μ l of plant extracts were combined with 290 μ l of DPPH. The plate was then incubated in the dark for 30 min. At 517 nm, absorbance was measured using a UV -Visible spectrophotometer. The absorbance of each sample was compared to that of a blank solution (DPPH without sample) to measure its free radical scavenging

activity. The positive control was ascorbic acid. The capacity to inhibit the DPPH radical was estimated as an inhibition percentage using the formula.

F. Determination of IC_{50}

The IC_{50} value (concentration required to scavenge 50 % of DPPH radicals) was calculated from linear regression analysis of percent inhibition versus concentration [19].

G. Statistical Analysis

All experiments were performed in triplicate and results were expressed as mean $\pm$ standard deviation (SD). Statistical analyses, including regression for IC_{50} calculation, were conducted using Microsoft Excel 2021.

III.RESULTS AND DISCUSSION

A. Scavenging Activity of ACHF-06

The antioxidant activity of ACHF-06 increased in a concentration dependent manner (Table 1). Low inhibition of 2.85 % produced at 20 μ g/mL, while a steady rise in scavenging potential was observed at higher concentrations i.e. 100 μ g/mL with highest inhibition 78.56 %. The IC_{50} value calculated from the regression curve was 73.03 μ g/mL, indicating moderate antioxidant strength. Triplicate absorbance readings showed low standard deviation (SD < 0.01), confirming experimental reliability.

B. Scavenging Activity of ACHF-07

ACHF-07 shows a clear concentration-dependent increase in antioxidant activity but was less potent compared to ACHF-06. At 20 μ g/mL, inhibition was minimal (0.26 %) and the highest inhibition at 100 μ g/mL reached 67.59 %. The IC_{50} value of 86.17

μ g/mL was higher than that of ACHF-06, indicating comparatively weaker radical-scavenging capacity.

ACHF-06 shows higher antioxidant activity, reaching 78.56 % inhibition at 100 μ g/mL with IC_{50} of 73.03 μ g/mL, whereas ACHF-07 achieved 67.59 % inhibition at the same concentration with an IC_{50} of 86.17 μ g/mL. A comparative evaluation revealed that ACHF-06 consistently exhibited higher DPPH inhibition than ACHF-07 at all tested concentrations. The lower IC_{50} value of ACHF-06.

The dose-dependent scavenging pattern observed for both formulations aligns with classical antioxidant behaviour,

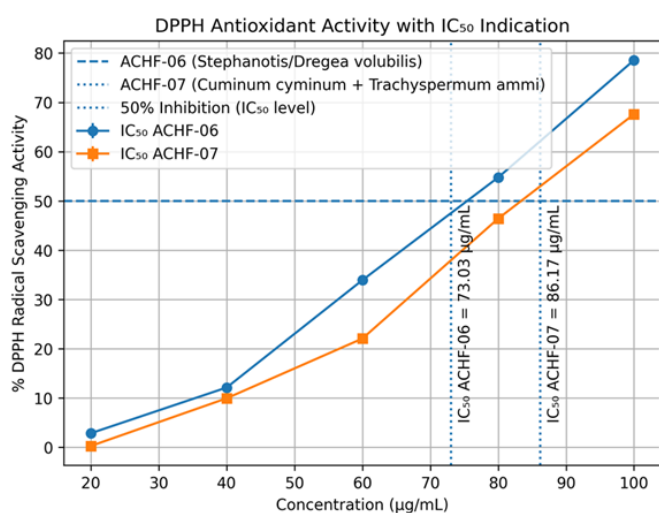
Overall, the results validate the traditional use of *S. volubilis*, *C. cyminum* and *T. ammi* as medicinal plants and support the use to enhance therapeutic efficacy.

IV.CONCLUSION

The comparative evaluation of two herbal formulations demonstrates that both ACHF-06 and ACHF-07 possess measurable free radical scavenging activity, suggesting the presence of antioxidant phytochemicals in each formulation. ACHF-06 exhibited stronger activity, reflected by its lower IC_{50} value and higher percentage inhibition across all tested concentrations. Although ACHF-07 displayed moderate antioxidant potential. However, ACHF-06 as a more effective natural antioxidant formulation and support the therapeutic relevance of herbal combinations in mitigating oxidative stress. These findings may contribute the traditional use of *S. volubilis*, *C. cyminum* and *T. ammi* as medicinal plants and support to the development of herbal antioxidant formulations for further pharmacological investigations at optimized concentration.

Table 1: Antioxidant activity of ACHF-06 and ACHF-07

Herbal Formulation	Conc. (µg/mL)	I	II	III	Average	Control	Inhibition %	SD	IC ₅₀ (µg/mL)
ACHF-06	20	0.983	0.971	0.978	0.97733	1.006	2.84957	0.00603	73.0327
	40	0.882	0.883	0.887	0.884	1.006	12.1272	0.00265	
	60	0.667	0.664	0.663	0.66467	1.006	33.9298	0.00208	
	80	0.453	0.457	0.455	0.455	1.006	54.7714	0.002	
	100	0.214	0.218	0.215	0.21567	1.006	78.562	0.00208	
ACHF-07	20	1.002	1	1.008	1.00333	1.006	0.26508	0.00416	86.1698
	40	0.906	0.908	0.904	0.906	1.006	9.94036	0.002	
	60	0.785	0.777	0.79	0.784	1.006	22.0676	0.00656	
	80	0.543	0.539	0.535	0.539	1.006	46.4215	0.004	
	100	0.341	0.317	0.32	0.326	1.006	67.5944	0.01308	

**Figure 1: DPPH radical scavenging activity of ACHF-06 and ACHF-07**

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