



# POLYHERBAL ELIXIR FORMULATION AND EVALUATION OF IT'S ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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**Abstract:** A synergistic approach to improving therapeutic efficacy and reducing side effects is provided by polyherbal medicines, which are made by combining several medicinal plants. They are extremely valuable in both traditional and modern medicine because of their varied phytochemical profiles, which allow for a wider range of biological activity. The ability of polyherbal formulations to target several pathways associated in complex diseases, including oxidative stress and inflammation, is the reason for their growing popularity. This study sought to determine the anti-inflammatory and antioxidant qualities of a polyherbal elixir made of *Terminalia arjuna*, *Asparagus racemosus*, and *Withania somnifera*. The study evaluated the effectiveness of the Elixir in reducing oxidative and inflammatory reactions using heat-induced protein denaturation assays and hydrogen peroxide scavenging. This study supports additional research towards the bioactive components of these herbs for possible therapeutic uses and offers a scientific basis for their traditional use in combination.

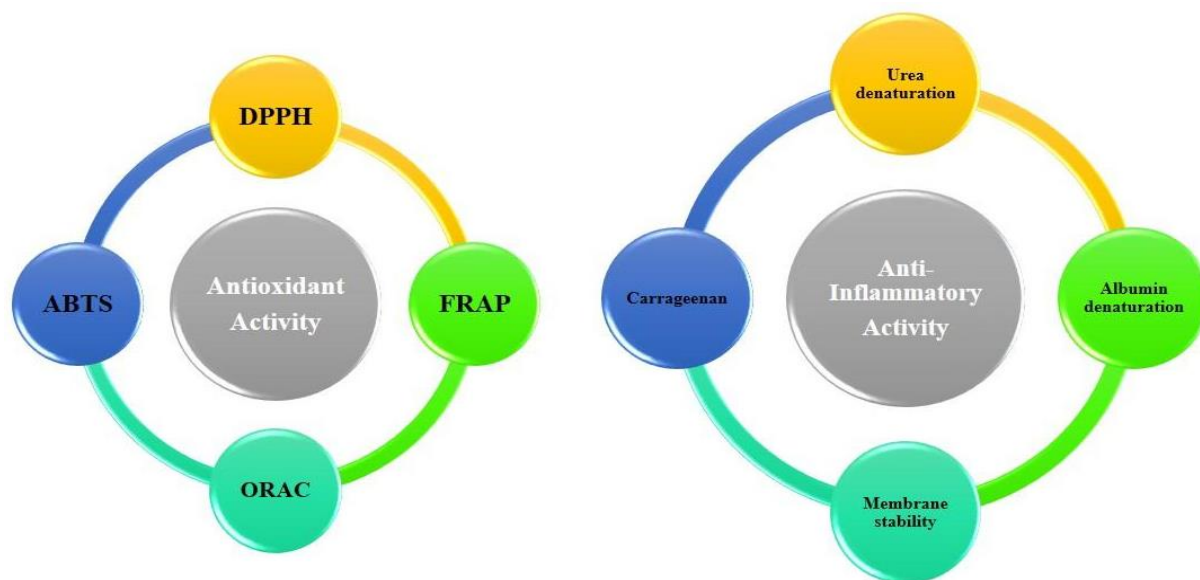
**Index terms:** Anti-inflammatory activity, Antioxidant activity, medicinal plants, Oxidative stress, Phytochemicals, Polyherbal formulation, protein denaturation.

## I. INTRODUCTION

The traditional Indian medical system known as Ayurveda emphasizes leading a healthy life and avoiding illness. It restores equilibrium and stops the recurrence of disease by using natural elements. India is a major biodiversity center with 15,000 medicinal plants, and 80% of the world's population uses traditional medicines. Since the active phytochemical constituents of individual plants are insufficient, polyherbalism is emphasized in Ayurvedic literature as a means of achieving greater therapeutic efficacy.<sup>(1)</sup> By lowering the dosages of individual herbs, polyherbal formulation (PHF) increases therapeutic efficacy and reduces side effects.<sup>(2)</sup>

Inflammation and oxidative stress are closely related mechanisms that play a major role in the development and course of a large number of chronic illnesses. Reactive oxygen species (ROS) are produced in excess of the body's antioxidant defenses, causing biochemical damage to accumulate in proteins, lipids, and DNA. This leads to inflammatory signaling cascades, including NF- $\kappa$ B and MAPK pathways, which further reinforce a self-reinforcing cycle of tissue damage.<sup>(3,4)</sup> Numerous illnesses are linked to this oxidative-inflammation axis, including metabolic diseases like type 2 diabetes and chronic kidney disease, neurodegenerative diseases like Alzheimer's and Parkinson's, and respiratory conditions like asthma and COPD.<sup>(5)</sup> Recent research indicates that redox-sensitive transcription factors, especially Nrf2, are essential for regulating the expression of antioxidant genes and reducing inflammatory damage, and that these pathways may be targets for treatment.<sup>(6)</sup>

Polyherbal formulations, which are composed of carefully selected extracts from multiple medicinal plants and enable a comprehensive assessment of their biological activity, offer a potent, multi-targeted strategy to fight oxidative stress and inflammation. Recent studies have shown that these formulations perform better than individual herbs in terms of anti-inflammatory qualities (albumin denaturation, membrane stability, carrageenan, or Freund's adjuvant-induced edema) and antioxidant capacity (DPPH, ABTS, and FRAP assays). For instance, the G-Immune Plus capsule (made from *Tinospora cordifolia*, *Withania somnifera*, *Emblica officinalis*, etc.) showed IC<sub>50</sub> values of 7–8 mg/mL in ABTS/DPPH testing and similar potency in membrane stabilization tests.<sup>(7)</sup> A different formulation (TC-16) that contained turmeric, ginger, black pepper, citrus, and honey showed synergistic antioxidant effects in tests like ORAC and  $\beta$ -carotene bleaching.<sup>(8)</sup> Meanwhile, paw edema, cytokine levels, and oxidative indicators in in vivo models (like Sprague-Dawley rats) were all markedly reduced by polyherbal therapy.<sup>(9,10)</sup> These findings demonstrate the medicinal potential of polyherbal blends and their measurable anti-inflammatory and antioxidant efficacy, providing a solid foundation for inclusion in research publications and further mechanistic studies.



**Figure 1: Types of In-Vitro Studies to Evaluate Antioxidant and Anti-inflammatory Activities**

Polyherbal elixirs are therapeutic formulations composed of multiple medicinal plant extracts, designed to work synergistically to enhance healing effects.<sup>(11)</sup> Unlike single-herb remedies, polyherbal elixirs combine diverse phytochemicals such as flavonoids, alkaloids, and phenolics that target multiple pathways, offering enhanced antioxidant, anti-inflammatory, and immunomodulatory benefits.<sup>(12)</sup> Traditionally used in Ayurvedic and other herbal systems, these elixirs are gaining renewed scientific interest for their potential in managing complex diseases linked to oxidative stress and chronic inflammation.

This study examines the anti-inflammatory and antioxidant properties of a polyherbal elixir made from specific medicinal plants with an established history of therapeutic benefit. Through in vitro experiments, the study seeks to determine how the combined phytoconstituents work in conjunction to reduce oxidative stress and regulate inflammatory responses. This study evaluates factors like the polyherbal elixir's ability to scavenge free radicals and inhibit inflammatory mediators, highlighting its potential as a natural remedy for oxidative and inflammatory diseases.



**Figure 2: Graphical representation of the Overall research**

## II. MATERIALS AND METHODS

### Herbal Raw Material Procurement:

The Arjuna (*Terminalia arjuna*) bark along with ashwagandha (*Withania somnifera*) and shatavari (*Asparagus racemosus*) roots are retrieved from the store following authentication by a botanist from CMR College of Pharmacy in Hyderabad, India.

### Chemicals required:

Ethanol, Hydrogen peroxide, Phosphate buffer, Ascorbic acid, Distilled water, Egg albumin, Salt, HCl, Diclofenac

**Materials:**

Test tubes, test tube holders, test tube stand, beaker, Volumetric flask (100ml capacity), measuring cylinder (50ml), butter paper, micropipette (1000 µg/ml), water bath, tripod stand, glass rod, thermometer, pH paper, mortar and pestle, pipette (10ml), personal protective equipment (gloves, goggles, lab coat).

**2.1 Preparation of Polyherbal Elixir:**

- **Plant material preparation:** To increase the surface area for extraction, crush or grind the dried bark and roots of specific herbs separately into a coarse powder.
- **Combining herbs:** You can make separate tinctures for each herb or mix them together in a particular proportion. Mix the powdered herbs in the desired ratio to create a combination. (5:1:5 of Shatavari, Ashwagandha, and Arjuna).
- **Menstrum:** Combine the distilled water and ethanol in a proportion that suits the strength of the tincture, such as 7:8.
- **Maceration:** Put the ground herbs in a suitable beaker and pour the menstrum over them, making sure the herbs are completely submerged. Place the beaker in a dark, cool place after sealing it.<sup>(13)</sup>
- **Steeping:** Shake the beaker every day while the mixture steeps for a few weeks. This will facilitate the extraction of the herbs' active ingredients.
- **Press and strain:** After the steeping time, pour the liquid into a clean container using filter paper. To extract as much liquid as you can, squeeze the marc (plant material).<sup>(14)</sup>
- **Filter and bottle:** If necessary, strain the tincture once more before transferring it to a tightly sealed glass beaker. Keep the tincture somewhere dark and cool.



Figure 3: Process of Tincture method of extraction

**III. EXPERIMENTAL PROCEDURE****3.1 In-vitro Evaluation of Antioxidant activity of Polyherbal Elixir**

**Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Assay:** The H<sub>2</sub>O<sub>2</sub> assay evaluates a plant extract's capacity to scavenge the hazardous reactive oxygen species hydrogen peroxide. By monitoring the decline in H<sub>2</sub>O<sub>2</sub> concentration following exposure, it illustrates the plant extract's antioxidant capacity. The scavenging capacity of the natural antioxidants in plant extracts has been thoroughly examined using the conventional UV method at 230 nm. In this experiment, test tubes containing the control, sample, and standard (ascorbic acid) were filled with 600 µL of 4mM H<sub>2</sub>O<sub>2</sub>. From a stock solution, the resultant elixir was made at various concentrations (20, 40, 60, 80, and 100 µg/mL). Phosphate buffer (0.2 M, pH 7.4) was then added to each test tube until the final volume reached 4 mL. Phosphate buffer, H<sub>2</sub>O<sub>2</sub>, and ascorbic acid were all present in the same ascorbic acid series, which was used as a reference (but without the sample). To enable the reaction to occur, each test tube was incubated for ten minutes at room temperature. To ensure reliability, the experiment was carried out in triplicate. The absorbance of H<sub>2</sub>O<sub>2</sub> in each solution was measured at 230 nm against the blank. Phosphate buffer acts as control/blank, which serves as a baseline with maximum oxidation of H<sub>2</sub>O<sub>2</sub>. The following formula is used to determine the percentage and to calculate IC<sub>50</sub> is given below:

$$\% \text{ scavenged (H}_2\text{O}_2\text{)} = (\text{A}_0 - \text{A}_1) / \text{A}_0 \times 100$$

Where, A<sub>0</sub> is the absorbance of the control, A<sub>1</sub> the absorbance of the sample.<sup>(15)</sup>



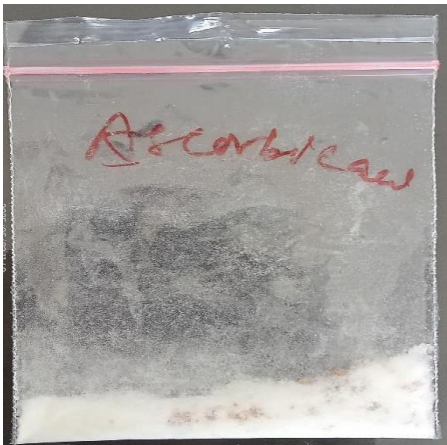


Figure 4: Ascorbic acid (Standard)



Figure 5: Standard solutions of different concentrations

Table 1: Standard drug: Ascorbic acid solutions preparation table

STANDARD	H <sub>2</sub> O <sub>2</sub>	ASCORBIC ACID	PHOSPHATE BUFFER
S <sub>1</sub> (20 µg/ml)	0.6ML	200µl	3.2ML
S <sub>2</sub> (40 µg/ml)	0.6ML	400µl	3ML
S <sub>3</sub> (60 µg/ml)	0.6ML	600µl	2.8ML
S <sub>4</sub> (80 µg/ml)	0.6ML	800µl	2.6ML
S <sub>5</sub> (100 µg/ml)	0.6ML	1000µl	2.4ML



Figure 6: Polyherbal Elixir (Sample)



Figure 7: Sample solutions of different concentrations

Table 2: Sample: Polyherbal Elixir solutions preparation table

STANDARD	H <sub>2</sub> O <sub>2</sub>	ELIXIR	PHOSPHATE BUFFER
T <sub>1</sub> (20 µg/ml)	0.6ML	200µl	3.2ML
T <sub>2</sub> (40 µg/ml)	0.6ML	400µl	3ML
T <sub>3</sub> (60 µg/ml)	0.6ML	600µl	2.8ML
T <sub>4</sub> (80 µg/ml)	0.6ML	800µl	2.6ML
T <sub>5</sub> (100 µg/ml)	0.6ML	1000µl	2.4ML

### 3.2 In-vitro Evaluation of Anti-inflammatory activity of Polyherbal Elixir

#### Egg Albumin (Heat-induced protein denaturation) Assay:

A popular technique for evaluating anti-inflammatory activity is the egg albumin assay, which determines a substance's capacity to prevent protein denaturation, particularly that of albumin, an indicator of inflammation. It focuses on how heat or stressors can cause proteins, especially albumin, to become denatured and how anti-inflammatory substances can prevent or lessen this process. In this experiment, test tubes holding the control, sample, and standard (Diclofenac) were filled with 0.45 mL of egg albumin solution. The final volume of the test tubes was filled with distilled water and serial dilutions of the standard (Diclofenac) and sample (Elixir) at concentrations of 10, 20, 30, 40, and 50 µg/mL. Small amounts of 1N HCl were added to the mixture to bring its pH down to the appropriate level. After 20 minutes of incubation at 37°C, the samples were heated for 30 minutes at 51°C while the temperature was closely watched. A Shimadzu UV-Visible spectrophotometer was used to measure the turbidity of the samples at 660 nm after they had cooled against control. Here distilled water is considered as control, which serves as baseline having maximum protein denaturation. To ensure accuracy, the experiment was carried out three times. The percentage inhibition of protein denaturation and IC<sub>50</sub> values were calculated and compared to the standard using the following formula:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{test}} \times 100}{\text{Absorbance}_{\text{control}}}$$

Where; Abs test is the absorbance of test sample,

Abs control is the absorbance of control reaction (having all reagents except the test sample.)<sup>(16)</sup>

**NOTE:** The concentration of a drug or compound needed to 50% inhibit a specific biological or biochemical process is known as the IC<sub>50</sub> value.

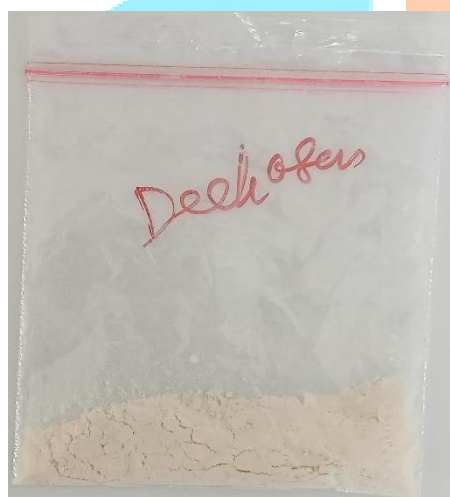


Figure 8: Diclofenac standard drug

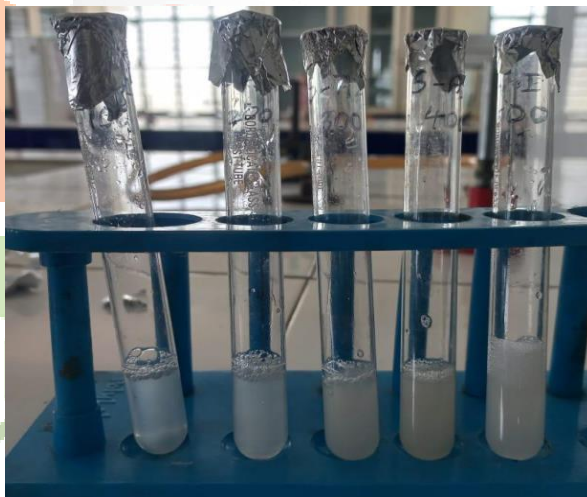


Figure 9: Standard solutions of different concentrations

Table 3: Standard drug: Diclofenac solutions preparation table

STANDARD	EGG ALBUMIN	DICLOFENAC	SOLVENT (D.W)	1N HCl
S <sub>1</sub> (10 µg/ml)	0.45ML	100µl	900µl	1ml
S <sub>2</sub> (20 µg/ml)	0.45ML	200µl	800µl	1ml
S <sub>3</sub> (30 µg/ml)	0.45ML	300µl	700µl	1ml
S <sub>4</sub> (40 µg/ml)	0.45ML	400µl	600µl	1ml
S <sub>5</sub> (50 µg/ml)	0.45ML	500µl	500µl	1ml



Figure 10: Polyherbal Elixir (Sample)



Figure 11: Sample solutions of different concentrations

Table 4: Sample: Polyherbal Elixir solutions preparation table

Standard	Egg albumin	Elixir	Solvent (D.W)	1N HCl
T <sub>1</sub> (10 µg/ml)	0.45ML	100µl	900µl	1ml
T <sub>2</sub> (20 µg/ml)	0.45ML	200µl	800µl	1ml
T <sub>3</sub> (30 µg/ml)	0.45ML	300µl	700µl	1ml
T <sub>4</sub> (40 µg/ml)	0.45ML	400µl	600µl	1ml
T <sub>5</sub> (50 µg/ml)	0.45ML	500µl	500µl	1ml



Figure 12: Preparation to heat solutions for protein denaturation

IV. RESULTS

4.1 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Assay:

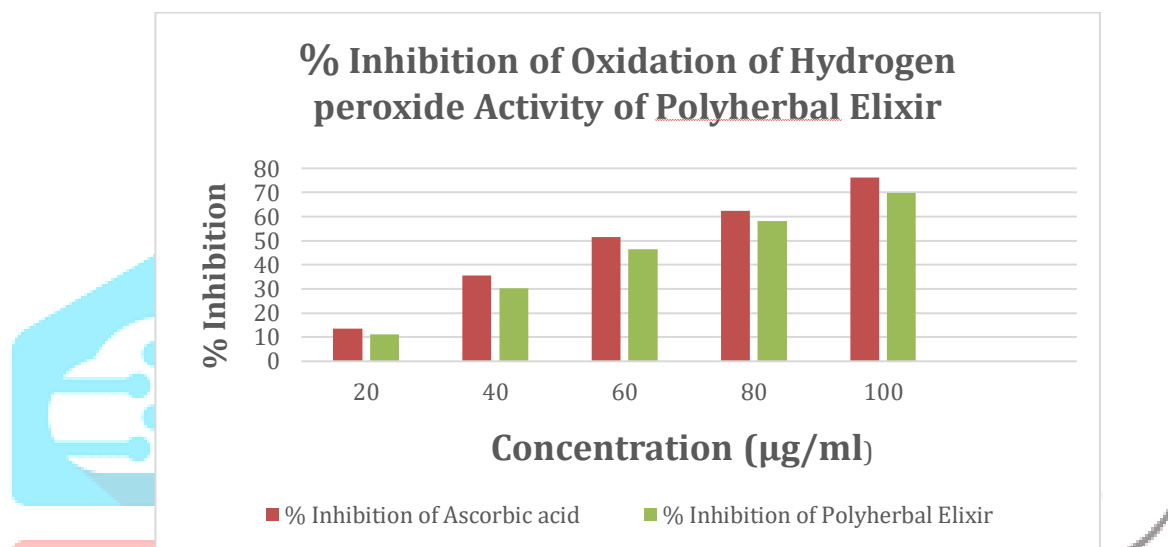
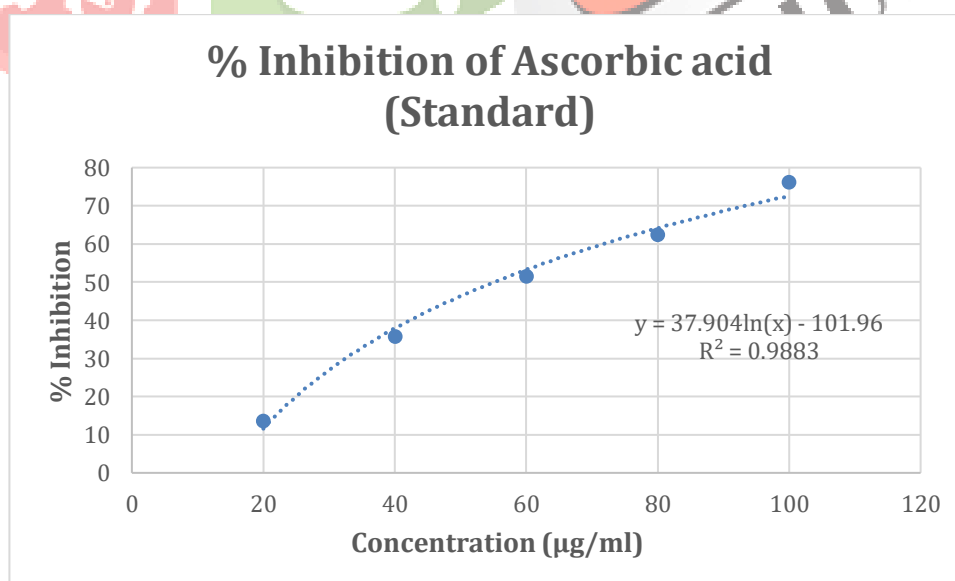
The Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assay, which assesses an antioxidant's capacity to scavenge H<sub>2</sub>O<sub>2</sub> and mitigate oxidative damage, was used to assess the test compound's antioxidant activity. The following are the outcomes of the H<sub>2</sub>O<sub>2</sub> assay:

Table 5: Effect of Ascorbic acid on oxidation of Hydrogen peroxide

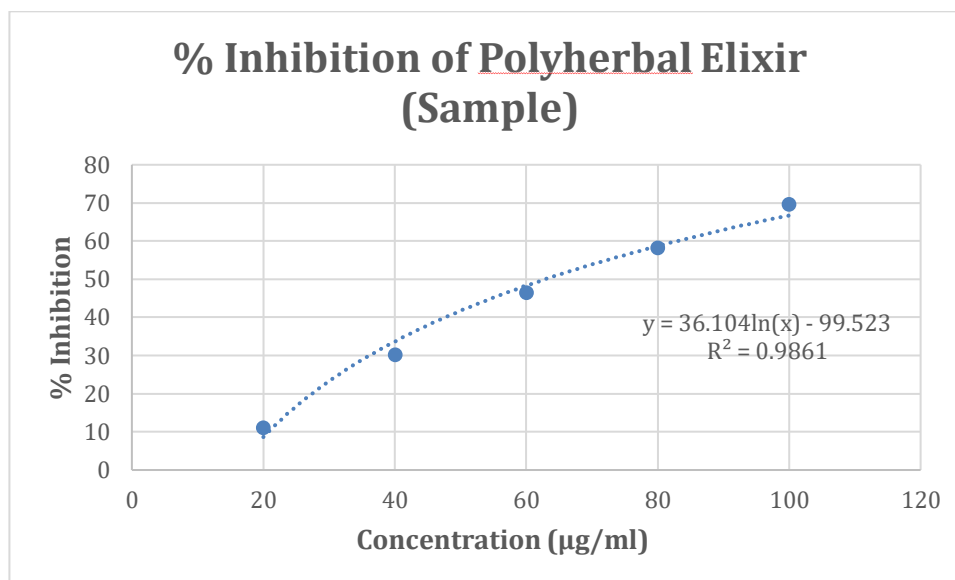
Concentration (µg/ml)	Control absorbance	Absorbance of Ascorbic acid	% Inhibition
20	0.86	0.743	13.6
40	0.86	0.553	35.67
60	0.86	0.416	51.56
80	0.86	0.323	62.4
100	0.86	0.205	76.16

**Table 6: Effect of Polyherbal Elixir on oxidation of Hydrogen peroxide**

Concentration (µg/ml)	Control absorbance	Absorbance of Polyherbal Elixir	% Inhibition
20	0.86	0.764	11.15
40	0.86	0.569	30.34
60	0.86	0.459	46.53
80	0.86	0.359	58.25
100	0.86	0.260	69.76

**Figure 13: Comparison of Ascorbic acid and Polyherbal Elixir on Antioxidant activity****Figure 14: IC<sub>50</sub> graph of Ascorbic acid (Antioxidant activity)**





**Figure 15: IC<sub>50</sub> graph of Polyherbal Elixir (Antioxidant activity)**

The IC<sub>50</sub> values of the Standard (Ascorbic acid) and Sample (Polyherbal Elixir) were found to be 55.09 µg/ml and 62.89 µg/ml.

#### 4.2 Egg Albumin Assay:

The egg albumin denaturation assay, which gauges an anti-inflammatory agent's capacity to prevent protein denaturation and stabilize proteins against heat-induced stress, was used to assess the test compound's anti-inflammatory activity. The following are the outcomes of the egg albumin assay:

**Table 7: Effect of Diclofenac on Heat Induced Protein denaturation**

Concentration (µg/ml)	Control absorbance	Absorbance of Diclofenac	% Inhibition
10	0.72	0.625	13.19
20	0.72	0.438	39.16
30	0.72	0.327	54.58
40	0.72	0.214	70.27
50	0.72	0.186	74.16

**Table 8: Effect of Polyherbal Elixir on Heat Induced Protein denaturation**

Concentration (µg/ml)	Control absorbance	Absorbance of Polyherbal Elixir	% Inhibition
10	0.72	0.647	10.14
20	0.72	0.506	29.72
30	0.72	0.421	41.54
40	0.72	0.336	53.33
50	0.72	0.270	62.5



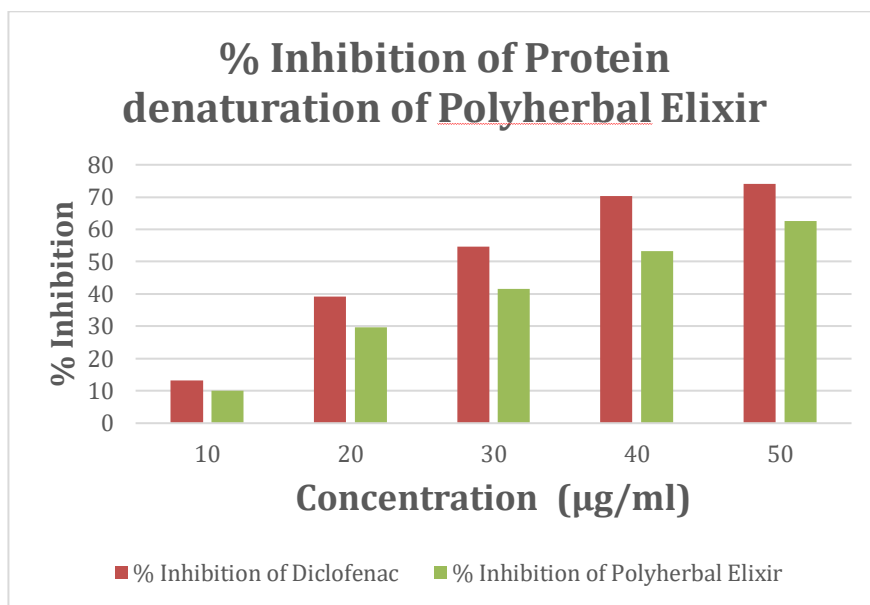


Figure 16: Comparison of Diclofenac and Polyherbal Elixir on Anti-inflammatory activity

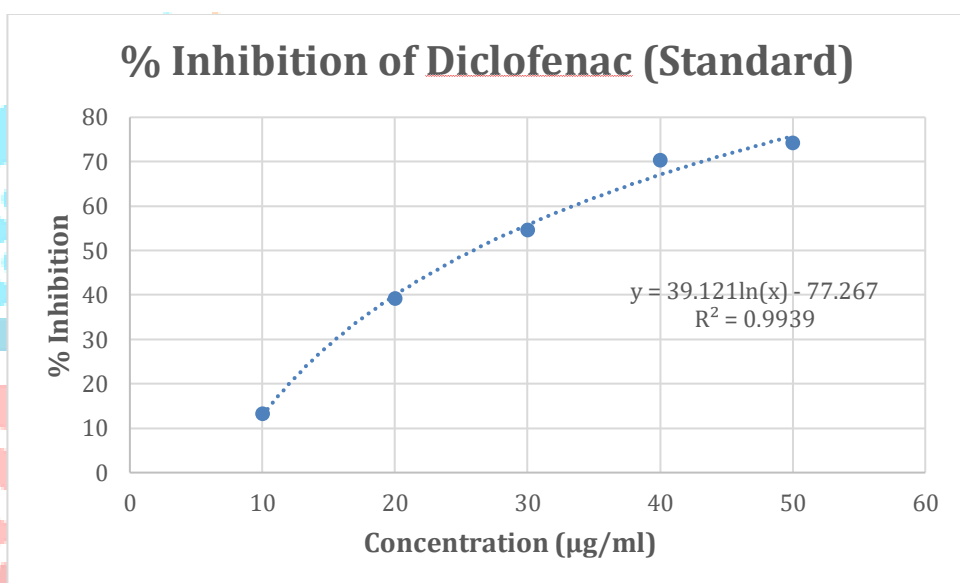


Figure 17: IC<sub>50</sub> graph of Diclofenac (Anti-inflammation activity)

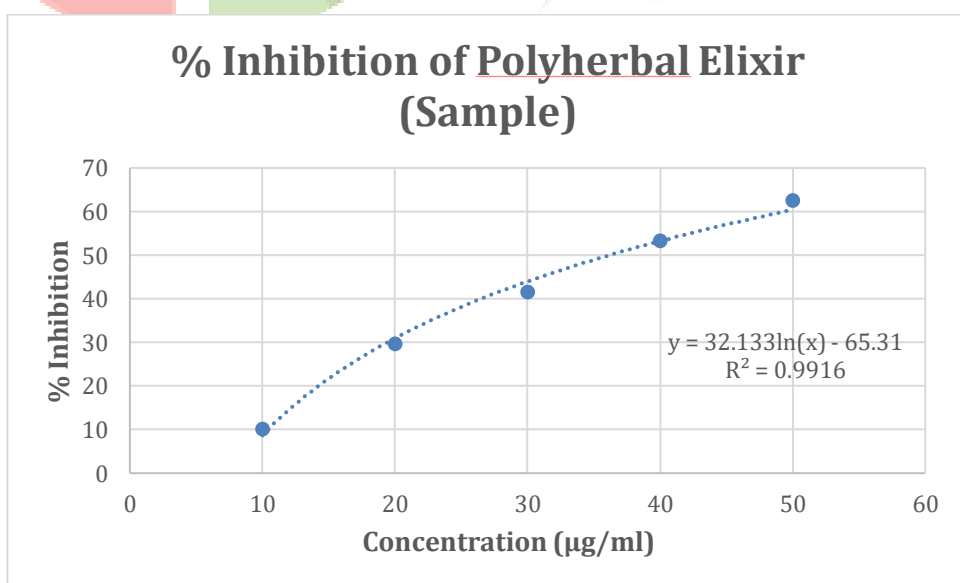


Figure 18: IC<sub>50</sub> graph of Polyherbal Elixir (Anti-inflammation activity)

The IC<sub>50</sub> values of the Standard (Diclofenac) and Sample (Polyherbal Elixir) were found to be 25.87 µg/ml and 36.179 µg/ml.

## V. DISCUSSION

### Inhibition of Oxidation of Hydrogen peroxide (Antioxidant activity)

One of the reactive oxygen species (ROS) and a major sign of oxidative stress is hydrogen peroxide ( $H_2O_2$ ), which can harm cells and tissues and play a role in a number of illnesses. By measuring a substance's capacity to lower hydrogen peroxide levels, the  $H_2O_2$  assay is a widely used technique to evaluate its antioxidant potential. Ascorbic acid, the standard antioxidant, showed notable scavenging activity in this assay, with an  $IC_{50}$  of 55.09  $\mu g/ml$  and 76.16% inhibition at 100  $\mu g/ml$ . Additionally, the Polyherbal Elixir demonstrated dose-dependent inhibition of hydrogen peroxide, attaining 69.76% inhibition at 100  $\mu g/ml$  with an  $IC_{50}$  of 62.89  $\mu g/ml$ . The Elixir showed significant antioxidant activity even though its higher  $IC_{50}$  value indicated that it had slightly less antioxidant potency than ascorbic acid. Because of their well-known ability to scavenge free radicals, bioactive phytochemicals like flavonoids, phenolic compounds, and saponins are probably responsible for this effect.

### Inhibition of Heat induced denaturation of proteins (Anti-inflammatory activity)

The inflammatory process depends heavily on protein denaturation, and a substance's potential anti-inflammatory qualities are indicated by its capacity to prevent heat-induced denaturation of albumin. In this case, protein denaturation was strongly inhibited by the common anti-inflammatory medication Diclofenac, which showed 74.16% inhibition at 50  $\mu g/ml$  with an  $IC_{50}$  of 25.87  $\mu g/ml$ . With an  $IC_{50}$  of 36.179  $\mu g/ml$  and a 62.5% inhibition at the same concentration, the Polyherbal Elixir also demonstrated a dose-dependent inhibitory effect. The Elixir demonstrated significant anti-inflammatory activity despite having a lower potency than Diclofenac, as indicated by its higher  $IC_{50}$ . Its high phytochemical content, especially flavonoids, saponins, tannins, and phenolic compounds, is probably responsible for this effect. These compounds are known to stabilize proteins and stop them from becoming denatured, which helps to reduce inflammation.

## VI. CONCLUSION

This study demonstrates the anti-inflammatory and antioxidant properties of a Polyherbal Elixir tincture extract that contains *Withania somnifera*, *Asparagus racemosus*, and *Terminalia arjuna*. The Elixir showed remarkable bioactivity through  $H_2O_2$  scavenging and egg albumin denaturation assays, with  $IC_{50}$  values of 36.179  $\mu g/ml$  for anti-inflammatory activity and 62.89  $\mu g/ml$  for antioxidant activity. Despite being fewer effective than the common medications Ascorbic acid (55.09  $\mu g/ml$ ) and Diclofenac (25.87  $\mu g/ml$ ), the Elixir's natural composition which is probably rich in flavonoids, tannins, and saponins supports its historical use in the treatment of inflammation and oxidative stress. These results imply that the Elixir is a good candidate for additional research, especially to identify its active ingredients to better understand how they work in Ayurvedic medicine.

## VII. ACKNOWLEDGEMENTS

We would like to acknowledge the CMR College of Pharmacy, Hyderabad, India for their support.

## VIII. CONFLICT OF INTEREST

There is no conflict of interest exists.

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