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POLYHERBAL GEL-BASED TREATMENT FOR RHEUMATOID ARTHRITIS FORMULATION, CHARACTERIZATION, AND EVALUATION

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ABSTRACT

Herbal plants such as *Carica papaya, Tamarindus indica, Calotropis gigantean, Cardiospermum halicacabum and Cympbopogan citratus* which have long been known to be a very important source of pharmaceutical properties. The study examined the anti inflammatory effect of polyherbal gel *in vitro*. Denaturation of proteins is the main cause of inflammation. The anti-inflammatory properties of polyherbal extract were studied at 500, 250, 100, 50 and 5 µg/mL using an albumin denaturation assay and proteinase inhibition assay. The free radical scavenging activity of polyherbal extract was evaluated by DPPH assay. The results showed that the anti-inflammatory activity of a gel based polyherbal extract shows a strong inhibition value at of IC 50 53.27 µg/ml, and Proteinase 156.2 µg/ml respectively. This study provides an evidence that the polyherbal gel possesses anti- inflammatory and antioxidant activity. That can be used for the treatment of Rheumatoid arthritis.

KEYWORDS: Polyherbal Gel-Based, anti inflammatory, and DPPH assay.

INTRODUCTION

Rheumatoid arthritis is considered one of the most common, and particularly attacks the joint causing significant individual and community burden. However, it can also attack other organs and cause extra-articular manifestations. These extra-articular manifestations include vasculitis, pulmonary involvement, and rheumatic nodules. Treatment modalities of rheumatoid arthritis have dramatically improved in recent years, with introduction of new management guidelines and diagnostic criteria. This revolution in management has caused significant decrease in long-term auricular and extra-auricular complications (Schneider and Kruger 2013).

Individuals with rheumatoid arthritis will suffer from significant comorbidities resulting from defects of the musculoskeletal system, and leading to physical functions decline, increased risk of long-term complications, and decreased quality of life. On the other hand, the community will also be affected by both the high medical expenses of management, and the reduced capacity of community members from the functional disability (Kahlenberg 2011).

Occupational therapist works with not only pain or symptoms management in rheumatoid arthritis but also the prevention of functional limitations and adaptation to lifestyle changes improve emotional state and social participation to maintain independence in daily living activities (Steultjens *et al*., 2004, Goodacre 2013). Arthritis includes approximately over 150 diseases and syndromes, and arthritis is the common problem and reason of substantial pain, limitation of activity, work, and social participation (Loyola *et al*., 2015, WHO 2016,). Rheumatoid arthritis is the most common variety among arthritis types (Macedo *et al*., 2009). Rheumatoid arthritis is typically diagnosed in adults between the ages 30 and 50, though it can develop at any age. Anticyclic citrullinated peptide protein (anti CCP) is especially used for early diagnosis. The most widely used and most reliable test, anti ccp2, was accepted as the gold standard in ACPAs in 2010. But ACPA is not positive for every rheumatoid arthritis patient (Smolen *et al*., 2016).

Oral administration of *Withenia somnifera* Linn., root powder showed the anti arthritic effect in adjuvant induced arthritic rats (Kadhim *et al*., 2016, Yaseri *et al*., 2016). Black pepper is indigenous and cultivated in South India. It is also cultivated in Indonesia, Brazil, Malaysia and Shrilanka. India ranks first in the cultivation of this drug. Pipper contains an alkaloid piperine, volatile oil, pungent resins, piperidine and starch. It is used as a aromatic, stimulant, stomachic and carminative. It increases the secreation of gastric juices. It also increases the

bio-availability of certain drugs. Piperine isolated from black pepper. Piperine administered orally at a dose of 20 and 100 mg/kg/day for eight days cause decrease in the arthritic symptoms in carrageenan-induced acute paw arthritis (Ubaid *et al*., 2017, Davis *et al*., 1986).

Artemisia absinthium L. (Asteraceae) in traditional Persian medicine, the aerial part of *A. absinthium* is one of the ancient drugs that possess medicinal effects on neuralgia, rheumatoid disorder, as well as inflammatory diseases. Scoparone, one of the main active constituents of *A. capillaris* Thunb., suppresses inflammatory cascade produced by macrophages significantly in IFN-cand LPS-stimulated RAW 264.7 cell mediated by reducing the release of NO and PGE2 106. Any decrease in the level of NO is mediated by inhibition of iNOS expression. Likewise, inhibition of COX-2 expression by scoparone has a pivotal role in reduction in inflammatory reaction mediators (Tripathy *et al*., 2010, Kim 2004). In a study conducted on gel based polyherbal formulation for the treatment of Rheumatoid arthritis.

MATERIALS AND METHODS

Collection of Plant Materials

The plant species *Carica papaya, Cymbopogon citratus, Cardiospermum halicacabum, Tamarindus indica, and Calotropis gigantea* were collected from Woraiyur, Trichy District, Tamil Nadu, India, located at 10.8308° N, 78.6799° E.

Phytochemical extraction (Soxhlet apparatus)

A total of 10 grams of crude powder from *Carica papaya, Cymbopogon citratus, Cardiospermum halicababum, Tamarindus indica,* and *Calotropis gigantea* were extracted using 200 milliliters of hydroalcoholic solution at a temperature range of 60°C to 80°C with a Soxhlet apparatus. After the extraction, the samples were evaporated using a rotary evaporator to retain the essential compounds.

Anti-inflammatory activity - Inhibition of albumin denaturation assay

Principle

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well documented cause of inflammation. As part of the investigation of the mechanism of the anti-inflammation

activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation.

Material Required

Acetylsalicylic acid, BSA was purchased from Sigma Aldrich, USA. 10X PBS was purchased from Himedia, India.

Procedure

Denaturation of proteins is the main cause of inflammation. Inhibition of protein denaturation was evaluated by the method of Mizushima and Kobayashi and Sakat *et al*. with slight modification. 500 µL of 1% bovine serum albumin was added to 500, 250, 100, 50 and 5 µg/mL of test sample. This mixture was kept at room temperature for 10 minutes, followed by heating at 51°C for 20 minutes. The resulting solution was cooled down to room temperature and absorbance was recorded at 660 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - (A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}) X100)$$

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Protease inhibition assay

Proteinases have been implicated in arthritic reactions. Neutrophils are reported to be a rich source of serine proteinases, which are localised in lysosomal granules. Leukocyte proteinases are involved in the development of tissue damage during inflammatory reactions and proteinase inhibitors provide substantial protection against this effect.

100 µL of 1% bovine serum albumin was added to 500, 250, 100, 50 and 5 µg/mL of test sample. This mixture was kept at room temperature for 5 minutes. The resulting solution was added with 250 µL of trypsin and followed by centrifugation at 6000 rpm for 10 minutes using centrifuge. Further supernatant was collected and absorbance was recorded at 210 nm. Acetyl salicylic acid was taken as a positive control. The experiment was carried out in triplicates and percent inhibition for protein denaturation was calculated using:

$$\% \text{ Inhibition} = 100 - ((A_{\text{control}} - A_{\text{test}}) / A_{\text{control}}) X100)$$

A dose response curve was plotted to determine the IC50 values. IC50 is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged.

Statistical analysis

The difference in estimated parameters between the groups was analyzed using one-way ANOVA with Bonferroni's test. Data expressed as mean ± SD. All parameters were analyzed at 95% confidence intervals and P value of <0.05 was considered to be statistically significant. Statistical analysis of the data was performed using Graphpad Prism version 6.00 for Windows, GraphPad Software, San Diego California USA.

Fig: 6 *Carica papaya.*

Fig: 7 *Cardiospermum halicambabum.*

Fig: 8 *Tamarindus indica.*

Fig: 9 *Cymbopogon citrates.*

Fig: 10 *Caloptropsis gigantean.*

Fig: 11. Albumin denaturation assay (Polyherbal gel)

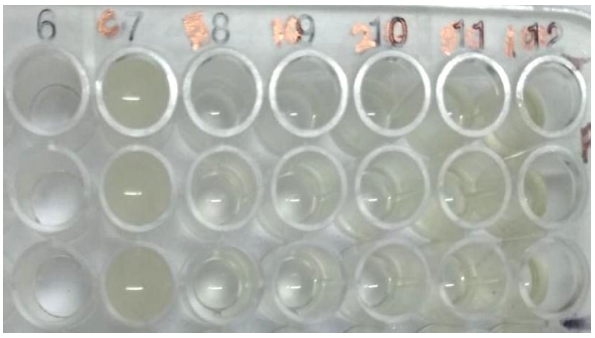

Fig: 12. Albumin denaturation assay (PVA)

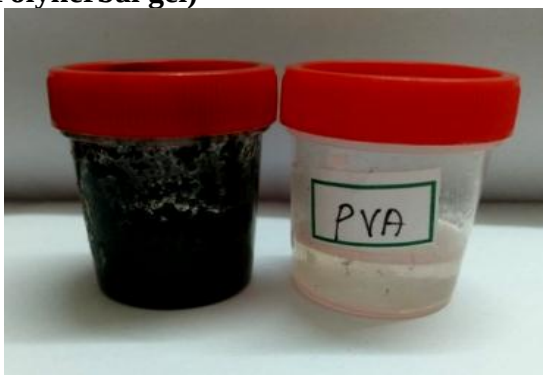

Fig: 13. Plant extract and PVA.

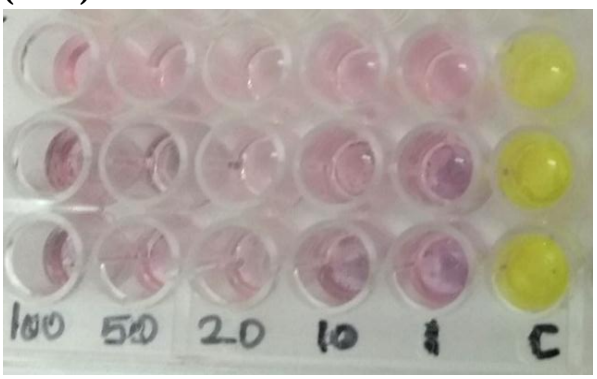

Fig: 14. Protease inhibition assay.

RESULTS AND DISCUSSION

Anti-inflammatory activity - Inhibition of albumin denaturation

The anti-inflammatory activity was evaluated using the albumin denaturation assay for both polyherbal formulations, along with phosphate-buffered saline (PBS) and polyvinyl alcohol (PVA). The results indicated that both formulations inhibited albumin denaturation in a concentration-dependent manner, with maximum inhibition observed at a concentration of 500 µg/ml. The IC50 values for the polyherbal formulation and PVA regarding albumin denaturation were determined to be 53.27 µg/ml and 81.72 µg/ml, respectively. According to Opie, tissue injury occurring during life may result from the denaturation of protein constituents in cells or intercellular substances. Therefore, a substance's ability to inhibit protein denaturation suggests its potential anti-inflammatory activity.

Anti-inflammatory properties of various plant extracts are available in the literature (Pranati *et al*., 2020). Overall, our study highlights the ability of the CTLA nanogel and the herbal formulation extract to effectively inhibit inflammation by preventing protein denaturation. The lysosomal membrane stabilization in activated neutrophils prevents the leakage of lysosomal contents like protease and other bactericidal enzymes, thereby playing a pivotal role in anti-inflammatory response in the human body. Red blood cells and lysosomal membranes of neutrophils have a similar structure, so stabilization of one membrane may

limit the destruction of the other. This principle is the basis for the human red blood cell membrane stabilization assay which uses hypotonicity induced lysis to evaluate the anti-inflammatory activity of drugs (Qamar *et al*., 2021).

Pande *et al*., 2015 reported that the phytosomes were prepared using the anti solvent precipitation method, which effectively combined phospholipids with drug extracts. The assessment of phytosomes demonstrated encouraging outcomes, with a substantial percentage yield of 72.5% and an entrapment efficiency of 86.94%±0.3. The phytosomal formulation demonstrated good stability and uniformity, as evidenced by a particle size of 158 nm and a zeta potential of -8 mV.

Protease inhibition assay

An anti-inflammatory assay using a proteinase inhibition method was conducted for a polyherbal formulation. The results demonstrated that the polyherbal formulation inhibited proteinase activity in a concentration-dependent manner, with the maximum inhibition of albumin denaturation occurring at a concentration of 500 µg/ml. The IC50 value for the proteinase inhibition assay was determined to be 156.2 µg/ml, as illustrated in the figures. This study highlights the promising properties of the polyherbal formulation gel, suggesting its potential use as a plant-derived remedy for gouty arthritis. Sakat *et al*., 2010 reported that the minimal adjustments, the inhibition protein denaturation approach was used. 1 mL (0.1 percent) bovine albumin fraction, 1 mL Tris-HCl buffer pH 7.8 solution, and 1 mL test solutions made up the reaction mixture (5 mL). The mixtures were incubated at 37°C for 20 minutes before being heated in a water bath at 70°C for 10 minutes for denaturation. Vandhana *et al*., 2025 have been reported that the Formulation F3's promising topical uses are highlighted by its outstanding performance in the gel matrix. Essential for successful cutaneous distribution, Formulation F3 displayed ideal viscosity and Spread ability in addition to high drug content (90.09%), appropriate pH and excellent homogeneity. Because of its extrudability, it is easy to administer and increases patient compliance, two important factors in patient care settings.

OD Value at 660 nm

Control Mean OD value: 2.315

Table 1: Albumin denaturation assay polyherbal formulation gel µg/ml.

S. No	Tested sample concentration (µg/ml)	OD Value at 660 nm (in duplicates)		
1.	Control	2.197	2.310	2.439
2.	500 µg/ml	0.655	0.821	0.648
3.	250 µg/ml	0.856	0.890	0.856
4.	100 µg/ml	0.567	0.807	0.831
5.	50 µg/ml	0.995	1.095	1.149
6.	5 µg/ml	1.446	1.277	1.343

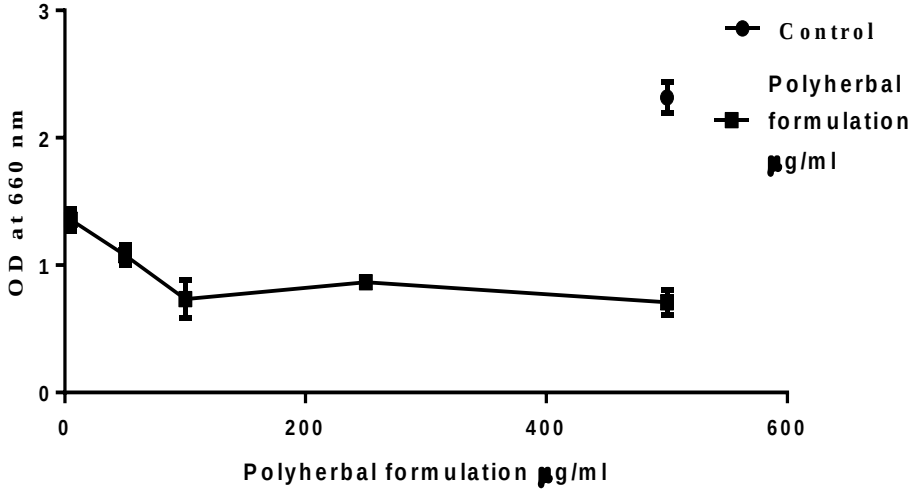

Fig. 15: Polyherbal formulation gel (µg/ml).

Table 2: Inhibition percentage of albumin denaturation (%).

S. No	Tested sample concentration (µg/ml)	Inhibition percentage albumin denaturation (%) (in triplicates)			Mean Value (%)
1.	Control	100	100	100	100
2.	500 µg/ml	71.70	64.53	72.00	69.41
3.	250 µg/ml	63.02	61.55	63.02	62.53
4.	100 µg/ml	75.50	65.14	64.10	68.24
5.	50 µg/ml	57.01	52.69	50.36	53.35
6.	5 µg/ml	37.53	44.83	41.98	41.44

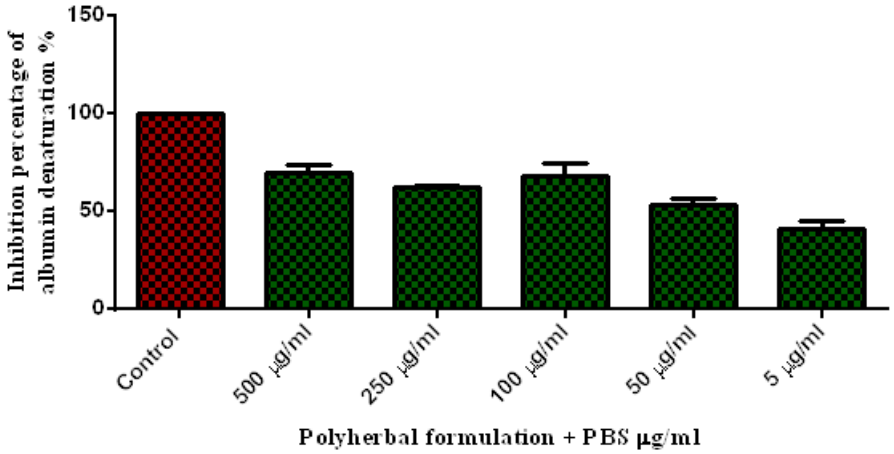

Fig. 16: Polyherbal formulation gel µg/ml.

Table 3: IC50 Value of tested sample: 53.27 µg/ml.

log(inhibitor) vs. normalized response -- Variable slope	Polyherbal formulation sµg/ml
Best-fit values	
LogIC50	1.726
HillSlope	-4.656
IC50	53.27
Std. Error	
LogIC50	0.03912
HillSlope	3.076
95% Confidence Intervals	
LogIC50	1.642 to 1.811
HillSlope	-11.30 to 1.988
IC50	43.85 to 64.71
Goodness of Fit	
Degrees of Freedom	13
R square	0.8294
Absolute Sum of Squares	3940
Sy.x	17.41
Number of points	
Analyzed	15

Albumin denaturation assay polyherbal formulation PVA µg/ml

OD Value at 660 nm

Control Mean OD value: 2.315

Table 4: Albumin denaturation assay polyherbal formulation PVA µg/ml.

S. No	Tested sample concentration (µg/ml)	OD Value at 660 nm (in duplicates)		
1.	Control	2.344	2.372	2.367
2.	500 µg/ml	0.634	0.669	0.414
3.	250 µg/ml	0.845	0.805	0.675
4.	100 µg/ml	0.799	0.832	0.675
5.	50 µg/ml	0.879	0.831	0.880
6.	5 µg/ml	0.984	1.289	0.913

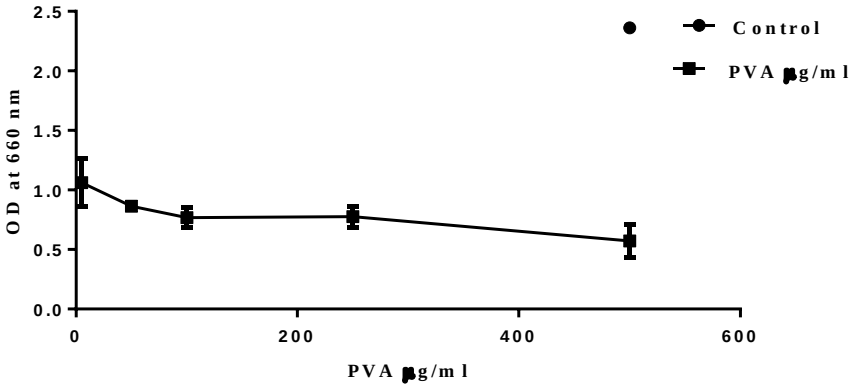

Fig. 17: Polyherbal formulation PVA µg/ml.

Table 5: Inhibition percentage of albumin denaturation (%).

S. No	Tested sample concentration (µg/ml)	Inhibition percentage albumin denaturation (%) (in duplicates)			Mean Value (%)
1	Control	100	100	100	100
2	500 µg/ml	73.14	71.66	82.46	75.75
3	250 µg/ml	64.21	65.90	71.41	67.17
4	100 µg/ml	66.15	64.76	71.41	67.44
5	50 µg/ml	62.77	64.80	62.72	63.43
6	5 µg/ml	58.32	45.40	61.32	55.01

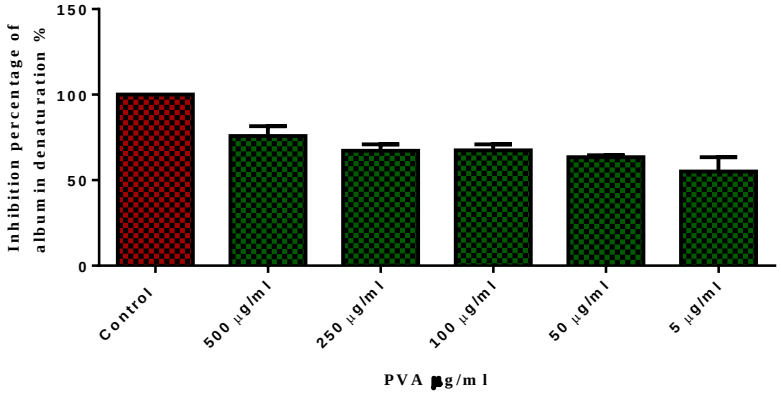

Fig. 18: PVA µg/ml.

Table 6: IC50 Value of tested sample: 81.72 µg/ml.

]	PVA µg/ml
Best-fit values	
LogIC50	1.912
HillSlope	-1.025
IC50	81.72
Std. Error	
LogIC50	0.1536
HillSlope	0.4215
95% Confidence Intervals	
LogIC50	1.580 to 2.244
HillSlope	-1.935 to -0.1143
IC50	38.06 to 175.5
Goodness of Fit	
Degrees of Freedom	13
R square	0.6428
Absolute Sum of Squares	7841
Sy.x	24.56
Number of points	
Analyzed	15

Protease inhibition assay

OD Value at 210 nm

Control Mean OD value: 1.998.

Table 7: Protease inhibition assay.

S. No	Tested sample concentration (µg/ml)	OD Value at 210 nm (in duplicates)		
1	Control	1.639	1.728	2.629
2	500 µg/ml	0.239	0.132	0.331
3	250 µg/ml	0.226	0.199	0.498
4	100 µg/ml	0.268	0.476	0.335
5	50 µg/ml	0.502	0.362	0.442
6	5 µg/ml	0.210	0.460	0.468

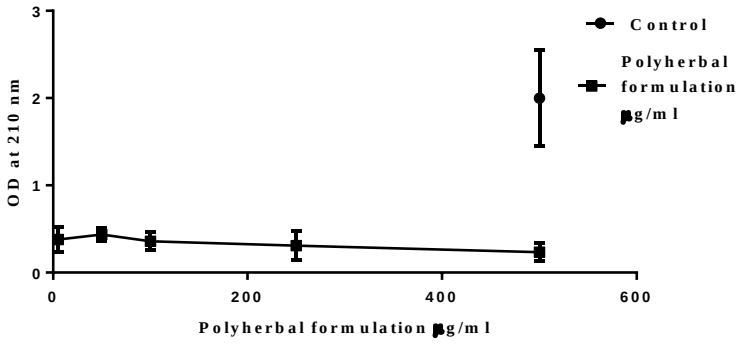

Fig. 19: Polyherbal formulation.

Table 8: Inhibition percentage of protease (%).

S. No	Tested sample concentration (µg/ml)	Inhibition percentage of protease (%) (in duplicates)			Mean Value (%)
1	Control	100	100	100	100
2	500 µg/ml	88.03	93.39	83.43	88.28
3	250 µg/ml	88.68	90.04	75.07	84.59
4	100 µg/ml	86.58	76.17	83.23	81.99
5	50 µg/ml	74.87	81.98	77.87	78.24
6	5 µg/ml	78.97	76.97	76.57	77.50

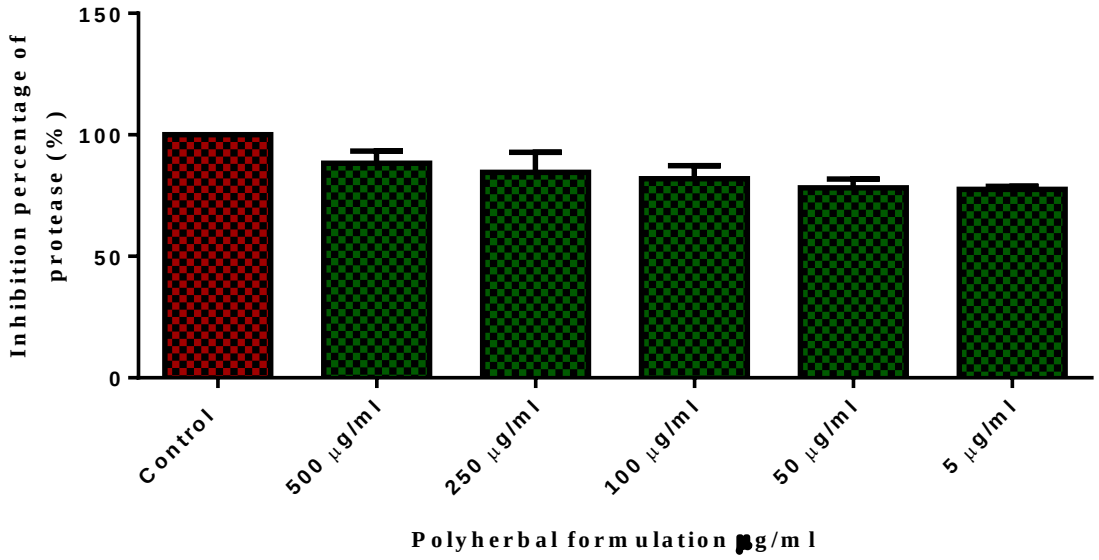

Fig. 20. Polyherbal formulation gel.

Table 9: IC_{50} Value of tested sample: 156.2 µg/ml.

log(inhibitor) vs. normalized response -- Variable slope	Polyherbal formulation µg/ml
Best-fit values	
LogIC50	2.194
HillSlope	-2.009
IC_{50}	156.2
Std. Error	
LogIC50	0.2236
HillSlope	1.712
95% Confidence Intervals	
LogIC50	1.711 to 2.677
HillSlope	-5.707 to 1.688
IC50	51.37 to 474.9
Goodness of Fit	
Degrees of Freedom	13
R square	0.2578

Absolute Sum of Squares	40239
Sy.x	55.64
Number of points	
Analyzed	15

CONCLUSION

Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease in which the immune system mistakenly attacks healthy cells in the body. This immune response leads to inflammation, characterized by painful swelling in the affected areas. While inflammation is a natural defense mechanism against foreign invaders, chronic inflammation can result in further damage in various conditions, such as cancer, allergies, asthma, autoimmune diseases, glomerulonephritis, hepatitis, inflammatory bowel disease, and rheumatoid arthritis, among others. The current study has demonstrated the anti-inflammatory and antioxidant properties of a polyherbal formulation through the albumin denaturation assay, revealing an IC50 value of 53.27µg/ml. Therefore, this polyherbal drug may be beneficial for the treatment of rheumatoid arthritis.

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