



## ***In Vitro* Anti-Oxidant and Anti-Arthritic Activity of Polyherbal Formulation (Capsules)**

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### ABSTRACT

The present study is aimed to evaluate antioxidant and anti arthritic activity of polyherbal formulation. The polyherbal formulation composed of six medicinal plants namely *Asparagus racemosus*, *Allium sativum*, *Bacopa monneri*, *Lippia nodiflora*, *Smilax zeylanica*, *Oldenlandia heynei*. anti oxidant activity by using Reducing power assay, Nitric oxide scavenging assay and anti-arthritic activity by using inhibition of protein denaturation, inhibition of protease enzyme activity, membrane stabilization. Diclofenac sodium was used as a standard drug.

**Key words:** Polyherbal formulation, anti-arthritis and anti- oxidant.

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## INTRODUCTION

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. It is a common disease having peak incidence in 3<sup>rd</sup> to 4<sup>th</sup> decades of life with 3-5 times higher preponderance in females<sup>1</sup>

Though conventional treatment options for this condition have improved in terms of effectiveness, the use of non steroidal anti-inflammatory drugs (NSAIDs) like etoricoxib, disease modifying anti- rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, leflunamide, hydroxychloroquine and corticosteroids like prednisolone, methylprednisolone have all been associated with adverse effects. Because of this reason, patients suffering from chronic musculoskeletal disorders are likely to seek alternative methods for symptomatic relief and are amongst the higher users of complementary and alternative medicine<sup>2</sup>. Recent studies show that many medicinal plants and herbal preparations are practiced for treatment of rheumatoid arthritis<sup>3</sup>. Herbal medicines widely used in health-care in both developed and developing countries are complex chemical mixtures prepared from plants and are limited in their effectiveness because they are poorly absorbed when taken orally<sup>4</sup>. According to an estimate of the World Health Organization (WHO), about 80% of the world population still uses herbs and other traditional medicines for their primary health care need<sup>5</sup>. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetic, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens. As per WHO definition, there are three kinds of herbal medicines: Raw plant material, processed plant material and Medicinal herbal products. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants the major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost<sup>6</sup>.

## MATERIALS AND METHODS

### Plant materials

The polyherbal formulation containing six medicinal plants such as *Asparagus racemosus*, *Allium sativum*, *Bacopa monneri*, *Lippia nodiflora*, *Smilax zeylanica*, *Oldenlandia heynei*. Were obtained from absa Herbals, Chennai. Samples were authenticated with help of Dr. P.Jayaraman, Director, Plant Anatomy & Research Centre, Tambaram, Chennai. All the other chemicals and reagents were pure analytical grade and obtained from local supplier. After QA approval the granules were filled in “0”-size capsules. The capsule net weight was 500 mg.

**Each capsule contains**

<b>Herbal drug</b>	<b>Quantity mg/capsule</b>
<i>Asparagus racemosus</i>	20 mg
<i>Allium sativum</i>	15 mg
<i>Bacopa monneri</i>	1.5 mg
<i>Lippia nodiflora</i>	110 mg
<i>Smilax zeylanica</i>	100 mg
<i>Oldenlandia heynei</i>	100mg

**Preparation of polyherbal extract<sup>7,8,9</sup>**

Polyherbal capsule granules were successfully extracted with hydro alcoholic mixture ( 3:2) for 12 hrs using soxhlet method. Then the hydro alcoholic extract was centrifuged twice at 3000 rpm for 5 min. Then the extract was subject to evaporated on water bath to dryness. The resulted dried extract was used for the *in vitro* anti oxidant and anti arthritic activity.

**IN VITRO ANTIOXIDANT ACTIVITY<sup>10,11</sup>****Reducing power assay**

Assay of Reducing Power was carried out by potassium ferricyanide method. 2.5 ml of various concentrations of test sample were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K<sub>3</sub>Fe (CN)<sub>6</sub>] (10g/L). The mixture was then incubated at 50°C for 20 minutes. To this mixture 2.5 ml of trichloroacetic acid (100g/L) was added, which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant solution was collected and mixed with 2.5 ml of distilled water and 0.5 ml FeCl<sub>3</sub> (1g/L) and absorbance was measured at 700 nm. Ascorbic acid was used as standard and phosphate buffer as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power.

$$\% \text{ Reductive ability} = \frac{\text{Absorbance of Control} - \text{Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

**Nitric oxide scavenging assay**

Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 M) in phosphate–buffered saline (PH 7.4) was mixed with 3ml of various concentrations of polyherbal formulation and incubated at 25°C for 150 min. The samples from the above were reacted with Griess reagent. The absorbance of the chromophore formed during the diazotization of nitrite with Sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546 nm. Ascorbic acid has taken as positive control.

$$\text{Nitric oxide Scavenging (\%)} = \frac{[(\text{Absorbance of Control} - \text{Absorbance of Sample})] \times 100}{(\text{Absorbance of Control})}$$

### **IN VITRO ANTI ARTHRITIC ACTIVITY**<sup>12,13</sup>

#### **Protein Denaturation Assay(PDA)**

The reaction mixture (5ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS,pH 6.4) and 2 ml of varying concentration of extract so that final concentration become 100,200,400,800µg/ml. similar volume of double distilled water served as control. Then the mixture were incubated at 37±2°C in a BOD incubator for 15 mins and then heated at 70°C for 5mins. After cooling, their absorbance was measured at 660 nm (SHIMADZU,UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (100,200,400,800µg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Percentage inhibition} = \frac{(\text{O.D. of Control} - \text{O.D. Sample})}{\text{O.D. of Control}} \times 100$$

#### **Inhibition of Protease enzyme Activity (IPA)**

The reaction mixture (2.0 ml) contains 0.06 mg trypsin, 1.0 ml of 25 mM Tris-HCl buffer (pH 7.4) and 1.0 ml Aqueous solution of test sample (100, 200, 400,800 mcg/ml) and were incubated at 37°C for 5 minutes. Then 1.0 ml of 0.8% (w/v) Casein was added and incubated for 20 minutes. 2.0 ml of 70% (v/v) Perchloric acid was added to terminate the reaction. The cloudy suspension was centrifuged. Optical density of the supernatant was read at 280 nm against buffer as blank. The percentage of inhibition was calculated using the following formula.

$$\text{Percentage inhibition} = \frac{(\text{O.D. of control} - \text{O.D. of sample})}{\text{O.D. of control}} \times 100$$

#### **Membrane Stabilizing Assay (MSA)**

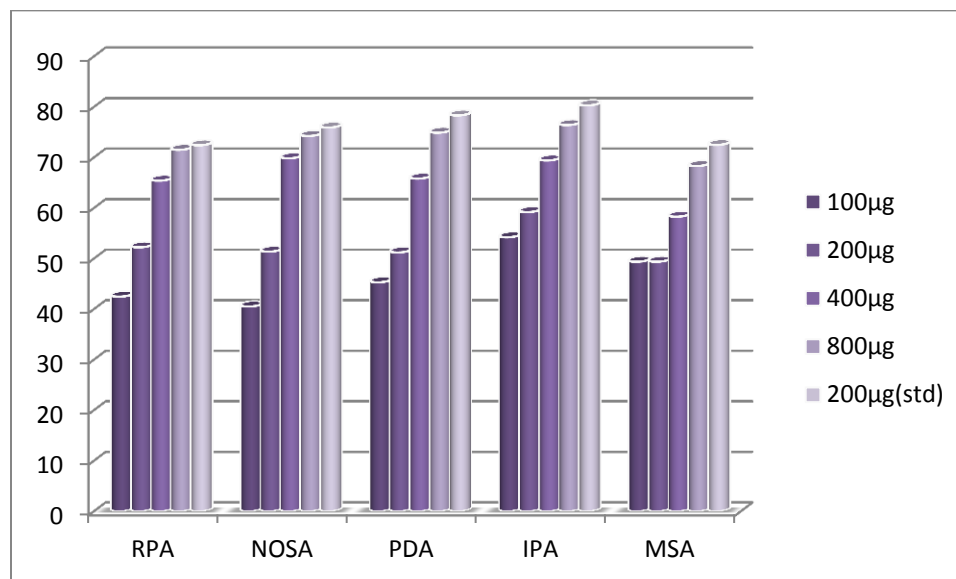
The reaction mixture (4.5 ml) consisted of 2 ml hypotonic saline (0.25% NaCl),1 ml 0.15 M phosphate buffer (pH 7.4) and 1 ml test sample (100, 200, 400 and 800 mcg/ml of final volume) in normal saline. 0.5 ml of 10% rat RBC in normal saline was added. For control sample, 1 ml of isotonic saline was used instead of test solution and sample solution without RBC act as product control. The mixture was incubated at 56° C for 30 minutes. The tubes were cooled under running tap water for 20 minutes. The mixtures were centrifuged at 2500 rpm for 5 min and the absorbance of the supernatant was read at 560 nm. Percentage membrane stabilizing activity was calculated as follows:

$$\text{Percentage Stabilization} = 100 - \frac{(\text{O.D. of test} - \text{O.D. of Product control})}{\text{O.D of Control}} \times 100$$

The result was compared with standard (diclofenac sodium 200 µg/ml) sample.

## RESULTS AND DISCUSSION

Anti oxidant and Anti-arthritic effect of polyherbal formulation was studied significantly by testing various *in vitro* parameters. Anti-arthritic effect of polyherbal formulation were compared to standard diclofenac sodium drug. The results were mentioned as graphical representation in Figure :1.



**Figure 1: Graphical representation of anti oxidant and anti arthritic activity of poly herbal formulation. RPA- Reducing power assay, NOSA- Nitric oxide scavenging assay, PDA- Protein denaturation assay, IPA- Inhibition of proteinase enzyme assay, MSA- Membrane stabilization assay, Std- standard drug**

The antioxidant activity of capsules showed a dose dependent inhibitory of reducing power assay and nitric oxide scavenging assay. The reducing power assay and nitric oxide scavenging assay was maximum at the concentration 800µg (71.53%, 74.27%, ) which was less than the standard drug, ascorbic acid 200 µg(72.43%,75.94%). The results were mentioned in Table 1.

There are certain problems associated with animal use in experimental pharmacology research such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. The production of auto antigen in certain arthritic disease may be due to denaturation of protein, Membrane lysis and proteinase action. Hence in the present study of the anti-arthritic activity of polyherbal capsule maximum percentage were observed (74.92%,76.43%,68.32%). The results were mentioned in (Table 2) from the results of our study reveals that extract of poly herbal formulation are capable of controlling the production of auto antigen and inhibit denaturation of protein, membrane lysis and proteinase action in rheumatic disease.

**Table 1. *In-vitro* antioxidant activity of Polyherbal capsule formulation**

Assay		100µg	200µg	400µg	800µg	200µg(std)
Reducing power assay		42.48±0.05	52.24±0.84	65.43±0.43	71.53±0.32	72.43±0.56
Nitric oxide scavenging assay		40.53±0.47	51.38±0.48	69.85±0.36	74.27±1.38	75.94±0.75

The values are expressed as mean ± SD. N=3

The reducing power assay and nitric oxide scavenging assay was maximum at the concentration 800µg (71.53%, 74.27%) which was less than the standard drug, ascorbic acid 200 µg(72.43%,75.94%).

**Table 2 In-vitro anti-arthritis activity of polyherbal capsule formulation**

Assay	Percentage inhibition at different concentrations				
	100µg	200µg	400µg	800µg	200µg(std)
Protein denaturation	45.32±0.42	51.23±0.12	65.84±0.84	74.92±0.43	78.34±0.56
Inhibition of protenase enzyme	54.23±0.22	59.21±0.45	69.43±0.67	76.43±0.47	80.32±0.34
Membrane stabilization	43.23±0.49	49.39±0.34	58.30±0.40	68.32±0.11	72.49±0.19

The values are expressed as mean ± SD. N=3 anti-arthritis activity of polyherbal capsule maximum percentage were observed (74.92%,76.43%,68.32%).

## CONCLUSION

From the results obtained in the present study it is concluded that, polyherbal formulation profound antioxidant activity through reductive ability and nitric oxide scavenging. It also showed potent antiarthritic activity.

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