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Original Article

Antioxidant and Antiarthritic Activity of Plant *Rivea Hypocrateformis* by *in-vitro* Methods

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ABSTRACT

Rivea hypocrateriformis is herb, which is traditionally used to cure various diseases. It contains flavonoids, tannins and phenolic compounds, which act as anti-inflammatory, antioxidant, anticancer, antiviral and antibacterial activities. Hence, the present study was undertaken with the objective of evaluating the anti-arthritic activity and antioxidant property of extract of *Rivea hypocrateriformis*. Extract of *Rivea hypocrateriformis was prepared* and analyse the antioxidant and antiarthritic activity by in-vitro model. The results obtained from DDPH method has been observed, IC50 of EERH was found TO be 71.25µg/ml, this study showed that the extract has a significant free radical scavenging activity. According to hydrogen peroxide scavenging method IC50 of EERH was 112.79µg/ml. Since phenolic compounds present in the plant extract are good electron donor, they may accelerate the conversion of hydrogen peroxide to water. It has been reported that the inhibition of protein denaturation by EERH was may be due to the presence of Flavanoids and tannins. The EERH showed 98.71% inhibition.

Keywords: *Rivea hypocrateriformis*, Antioxidant, Anti-arthritic activity, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ethanolic extract of *Rivea hypocrateriformis* (EERH)

INTRODUCTION

Now a day's majority of diseases are because of the shift in the balance of pro-oxidant and antioxidant. Pro-oxidant conditions dominate either due to the increased generations of the free radicals caused by excessive oxidative stress of the current life. Plants especially fruits and vegetables are known to possess phytochemicals such as flavonoids and vitamins that posses antioxidant activity and that can be used to scavenge the excess free radicals from human body [1,2]. Natural antioxidants includes many biological functions, degenerative diseases, include protection against oxidative stress

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and are reported to possess antiviral, antibacterial, anticancer, antiaging, anti‑inflammatory, antiallergic and hepatoprotective properties [3-5]. Therefore, the evaluation of antioxidant activity of various herbal products that were identified by Ayurveda system and used till date, are required for the identification of their capability to scavenge the free radicals [6,7].

Though there are so many methods for evaluate antioxidant property, 1,1-diphenyl−2-picrylhydrazyl (DPPH), free radical scavenging method, reducing power method, xanthine oxidase method. Phosphomolybdenum method used to determine the total antioxidant capacity, based on the reduction by the antioxidant compounds and the formation of a green complex [8]. It has been studied that the antioxidant property of the plants may be due to the presence of phenolic compounds[12,14,15]. Many research demonstrated that phenolic and flavonoid compounds of plant *Rivea hypocrateriformis* act as reducing agents, which exhibit their antioxidant property [9-11]. Therefore, the present work aims to investigate antioxidant activity of plant extract *Rivea hypocrateriformis* through the use of in vitro assay [12-14]

The pre-eminent leniency in rheumatoid arthritis population is entirely attributed to cardiovascular adversity. These patients suffer more myocardial infarction, cerebrovascular accidents and even heart failure than those without rheumatoid arthritis [15-19]. Etiology is not clearly known, it occurs in immunogenetically predisposed individuals to the effect of microbial agents acting as trigger antigens or due to the existence of infectious agents lack as mycoplasma, Epstein barr virus (EBR) cytomegalovirus (CMV) or rubella. Records and symptoms are swelling and pain of joints in symmetrical fashion.

Onset of disease begins with fatigue, weakness, morning stiffness, arthralgia, mylagia, redness and difficult movement. Rheumatoid nodules also seen close to the joints of neck, shoulder, elbow, hip, knees and ankles. NSAIDS were used first to afford symptomatic relief from pain, swelling, morning stiffness, immobility, but do not arrest the disease. *Rivea* hypocrateriformis (family: convolvulaceae) posses antiarthritic activity. [20, 21]

Leaf and young shoots of plant are eaten as vegetables. Juice of leaf is used for skin diseases of hair and scalp. Juices of leaves along with babul twig and sugar is taken with cow's milk for relief from rheumatic pain [19, 20], so the purpose of present study is to evaluate the antiarthritic activity of plant extract *Rivea hypocrateriformis* [22-24].

MATERIALS AND METHODS

Collection of plant: The plant *Rivea hypocrateriformis* choisy (Desr) was collected from the Bharathiyar university campus at Coimbatore during the month of July and it was authenticated by Taxonomist.

Extraction of plant: The leaves were washed thoroughly and dried in shade. The shade dried leaves were reduced to coarse powder (Sieve No. 10/40) and then subjected to the following phytochemical screening tests. The dried powder material of *R. hypocrateriformis* was extracted successively by ethanol for 48 h using Soxhlet apparatus. The extract was stored in refrigerator for further studies.

In-vitro antioxidant activity

Diphenyl picryl hydrazyl (DPPH) method:

DPPH is a stable free radical with a distinctive ESR signal. Its reaction with antioxidants can be followed by the loss of absorbance at 517nm. It is widely accepted that DPPH accept an electron or hydrogen radical and become a stable diamagnetic molecule. Due to its odd electron, the ethanol solution of DPPH (purple colour solution) shows a strong absorption at 517nm. DPPH radicals react with suitable reducing agents where the pairing of electrons takes place and the solution loses colour stochiometrically with the number of electrons taken up [25, 26].0.1mM diphenyl picryl hydrazyl in ethanol is used a reagent. A stock solution of DPPH was prepared in ethanol. To the test samples of different concentrations of extracts, 4mL of DPPH was added. Control without test compound was prepared in an identical manner. Blank was prepared in the similar way, where DPPH was replaced by ethanol. The reaction was allowed to be completed in the dark for about 30min. Then the absorbance of test mixtures was read at 517nm. The percentage inhibition was calculated and expressed as percent scavenging of DPPH radical. Vitamin C was used as standard. The percentage scavenging was calculated using the formula. The concentration of the sample required for 50% reduction in absorbance (IC_{50}) was calculated using linear regression analysis.

% Inhibition = [(Control-Test)/Control] x 100

Determination of scavenging activity against hydrogen peroxide: The radical scavenging activity against hydrogen peroxide of plant extract was determined by using the method of Ruch *et al.* The principle based on the capacity of the extracts to decompose the hydrogen peroxide to water [27, 28]. 6.0% hydrogen peroxide diluted with water in the ratio of 1:10 with 0.1M, pH 7.4 phosphate buffer used as reagent. The ethanolic extract of

Rivea hypocrateriformis was dissolved in ethanol to get a stock of 1mg/ml. Varying quantities of the stock solution were added to 3.8ml of 0.1M phosphate buffer solution (PH 7.4) and then mixed with 0.2ml of hydrogen peroxide solution. The absorbance of the reaction mixture was measured at 230nm after 10min.The reaction mixture without sample was used as blank. Ascorbic acid was used as standard. The percentage inhibition of hydrogen peroxide was calculated using the formula;

%Inhibition = (A control- A sample)/A control x 100

***In-vitro anti-arthritic activity*:** Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Anti arthritic activity was studied by inhibition of protein denaturation method [29-32]. EERH (Ethanolic extract of *R. hypocrateriformis*) is taken as test extract and Diclofenac sodium as Standard Drug. The test solution consists of bovine serum albumin (5% w/v aqueous solution) with different concentrations 100, 200, 400, 800, 1000 µg/ml of EERH was prepared. The test control solution was prepared which consists of bovine serum albumin and distilled water. The product control consists of distilled water and test solution (of different concentrations) and standard solution consists of bovine serum albumin and of Diclofenac sodium solution (of different concentration). All the above test samples were adjusted to pH 6.3. They were incubated at 37^0C for 20 minutes and heated at 57^0C for 3 minutes. Allow cooling and small quantity of phosphate buffer (pH 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula;

Percentage Inhibition = 100 – [{(optical density of test control – optical density of product control)/ optical density of test solution} × 100

Percentage inhibition = 100 - $\frac{\{(OD \text{ of test solution} - OD \text{ of product control})\}}{OD \text{ of test control}}$

RESULTS

Diphenyl picryl hydrazyl (DPPH) method:

According to this method, test drug had better antioxidant activity when compared with standard drug. The details of their respective IC_{50} values are illustrated in Table 1(Fig. 1).

Table 1: Free radical scavenging activity of EERH by DPPH Assay

S. No	Conconcetration (µg/mL)	Percentage inhibition by standard ascorbic acid	Percentage inhibition by EERH
1	20	25.86 ± 0.61	36.59 ± 0.01
2	40	53.32 ± 0.45	45.10 ± 0.06
3	60	62.20 ± 0.38	52.16 ± 0.62
4	80	81.21 ± 0.71	54.90 ± 0.06
5	100	93.73 ± 0.41	52.31 ± 0.55
	IC_{50}	47.04 **µg/mL**	71.25 **µg/mL**

Value obtained from regression lines with 95% of confidence level. IC50 is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiment at S.D. (5%) for the above table Values are expressed as means ± standard errors.

Table 2: Free radical scavenging activity of EERH by Hydrogen peroxide method

S. No	Concentration (µg/mL)	Percentage inhibition by standard Ascorbic acid	Percentage inhibition by EERH
1	20	18.81 ± 0.12	16.23 ± 0.15
2	40	23.52 ± 0.28	24.81 ± 0.50
3	60	47.85 ± 0.11	31.40 ± 0.29
4	80	72.18 ± 0.54	37.98 ± 0.18
5	100	86.56 ± 0.39	40.89 ± 0.37
	IC_{50}	47.04 **µg/mL**	112.79**µg/mL**

Value obtained from regression lines with 95% of confidence level. IC50 is defined as the concentration sufficient to obtain 50% of a maximum effect estimate in 100%. All values given are mean of triplicate experiment at S.D. (5%) for the above table Values are expressed as means ± standard errors.

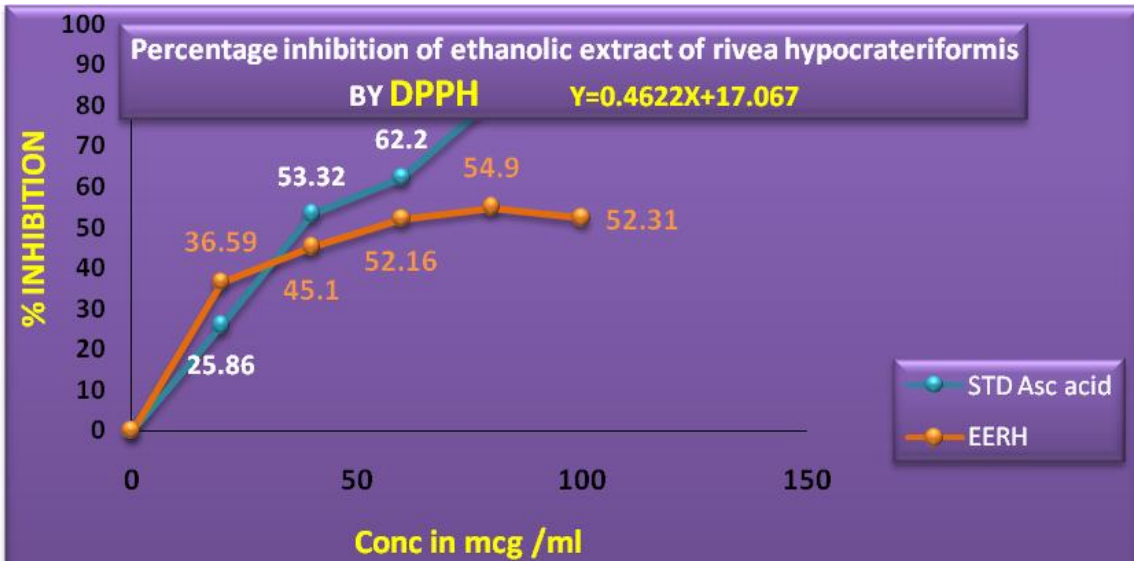

Fig 1: Free radical scavenging activity graph of EERH by DPPH method

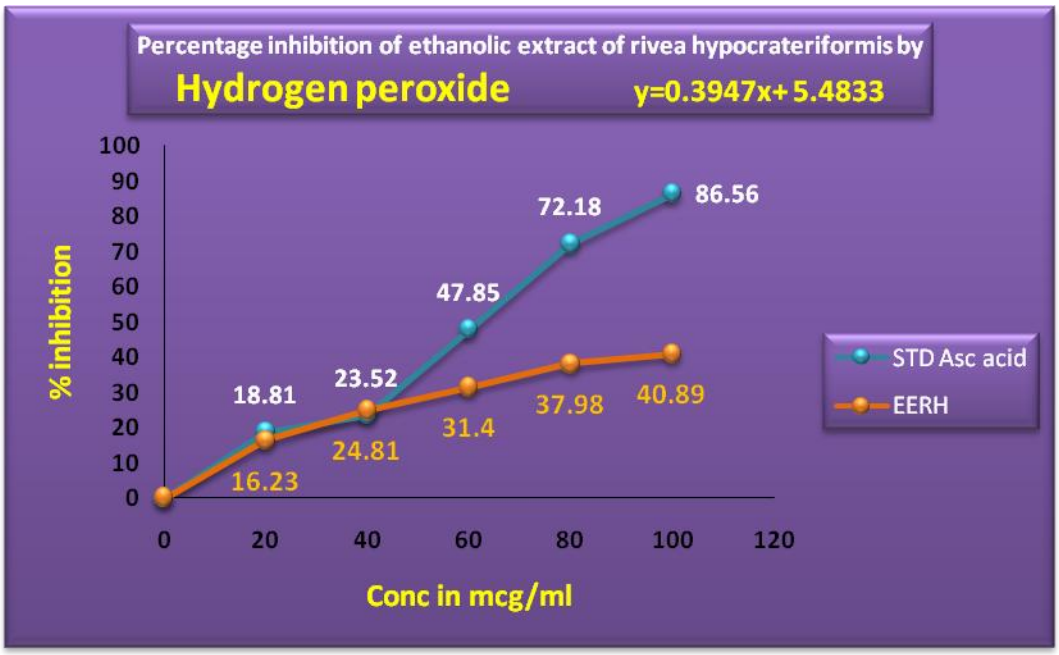

Fig 2: Free radical scavenging activity graph of EERH by Hydrogen peroxide method

***In-vitro anti-arthritic activity*:** The control represents 100% protein denaturation. The results were compared with the standard drug, diclofenac sodium percentage inhibition (table 4) and found that EERH. The percentage inhibition of protein denaturation by EERH was compared with standard drug Diclofenac sodium (Fig 3) and shows 100 % protein denaturation.

Table 3: Inhibition of Protein Denaturation by the Effect of ethanolic extract of *Rivea hypocrateriformis*

S. No	Conc. of Test solution(µg/ml)	% Inhibition of protein denaturation
1	100	60.99
2	200	69.04
3	400	72.23
4	800	80.33
5	1000	98.71

Table 4: Inhibition of Protein Denaturation by the effect of standard solution

S.No	Conc. of standard solution(µg/ml)	% Inhibition of protein denaturation
1	100	63.96
2	200	72.89
3	400	80.63
4	800	83.99
5	1000	99.00

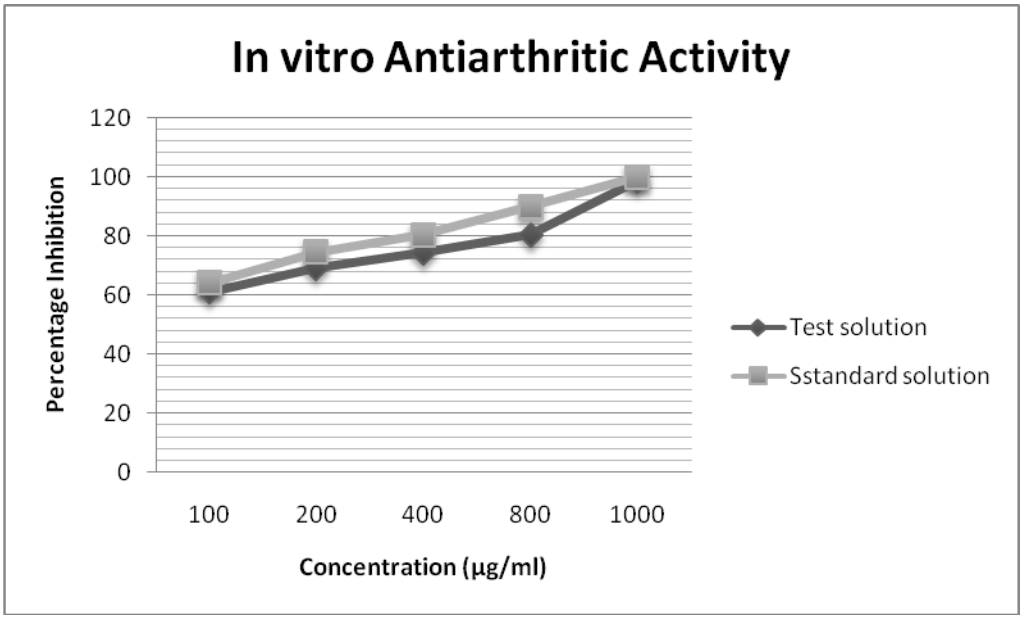

Fig 3: In-vitro anti-arthritic activity of EERH

DISCUSSION

From the results obtained by using DPPH method, it has been observed that the percentage inhibition of extract was 52.31% and for standard ascorbic acid 93.73% at a concentration of 100µg/ml (showed in the fig-1). IC_{50} of EERH was found to be 71.25µg/ml. Thus this study showed that the extract has a significant free radical scavenging activity. It has been shown that the IC_{50} of EERH was 112.79µg/ml compared to IC_{50} of standard ascorbic acid 47.04µg/ml (showed in the fig-2 & Table-2). Since phenolic compounds present in the plant extract are good electron donor, they may accelerate the conversion of hydrogen peroxide to water. This part of the pharmacological study deals with the in vitro anti arthritic activity of EERH.

The principle involved is the inhibition of protein denaturation. Denaturation of protein was found to be one of the causes of rheumatoid arthritis. In rheumatoid arthritis the auto antigen production may be due to denaturation of protein. The mechanism of denaturation involves the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding. It has been reported that anti arthritic activity may be due to the presence of phenolic compounds. The inhibition of protein denaturation by EERH was may be due to the presence of flavanoids and tannins. The EERH showed 98.71% inhibition at 1000 µg/ml and when compared to standard drug Diclofenac sodium, it showed almost same 99 % inhibition.

At the end of this study, the plant *Rivea hypocrateriformis* contains some groups of chemical constituents possessing antioxidant and anti-arthritic potential which was evaluated by *in vitro* and *in vivo* studies. The results displayed that *Rivea hypocrateriformis* had 100% protein denaturation inhibition showing its antiarthritic activity and inhibition of free radicals showed its antioxidant. These results confirm that the utilization of plant *Rivea hypocrateriformis* can be a potential drug for the treatment of arthritis.

CONCLUSION

Arthritis affects people globally and men are 3 times more prone to it than woman. The onset is generally between 40 - 60 years of age although it can occur at any age. There are many children under the age of 16 with the juvenile form of the disease. Other than genetic dispositions, exact external cause is not known but cigarette smoking and environmental pollutants are important precipitating factors. So far, there is no cure for it,

but understanding about the inflammatory process helps to manage it effectively The good news is that the prognosis today, if diagnosed and treated early, is significantly better than it was 20-30 years ago and many people have a much better quality of life in spite of having arthritis. Herbal drugs and its isolated constituents can play vital role in the management of arthritis.

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