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In-vitro anti-arthritic and anti-platelet activity of *Galinsoga parviflora* Linn.

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Abstract
The present study is objected to evaluate the methanolic leaf extract of *Galinsoga parviflora* for acclaimed anti-arthritic activity using *in-vitro* inhibition of protein denaturation model as well as as anti-platelet activity. Acetyl salicylic acid (Aspirin) was used as a standard drug. Phytochemical analysis of methanolic extract of *Galinsoga parviflora* has indicated the presence of steroids, flavonoids and terpenoids types of compounds. Since these compounds are of pharmacological interest, coupled with the use of this plant in traditional medicine, i.e. for possible anti-arthritic as well as anti-platelet activity. Results revealed that the methanolic extract of *Galinsoga parviflora* at three different concentrations (100,250 and 500 mcg/ml) possessed significant anti-arthritic well as anti-platelet activity as compared to standard used drug acetyl salicylic acid. The plant extract showed dose dependent activity.

Keywords: *Galinsoga parviflora*, anti-arthritic activity, anti-platelet activity, protein denaturation

Introduction
The immune system is a well organized and well regulated system. The deregulation of the immune system may lead to development of autoimmune diseases. Rheumatoid arthritis is an destruction of articular cartilage. It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female. Its prevalence depends upon age. Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas. Number of synthetic medicines has been derived from medicinal herbs. *Galinsoga parviflora*, is a quick weed, grows to a height of 75cm. The leaves are ovate or narrowly ovate, 2-5cm long, involcral bracs 2-3mm long, ray florets white, 5 per head, rarely pink, 3-toothed 1-2mm long, pappus of ray florets absent or very reduced. Achenes sparsely appressed pubescent or glabrous. The phytochemical investigstion of *Galinsoga parviflora* shows the presence of steroids, flavonoids and terpenoids and volatile constituents. Extract of leaves with salt is also useful in fever, diarrhea and snake bite.

Materials and Methods
Plant material:
The leaves of plant *Galinsoga parviflora* were collected from local area of Bhopal, M.P., India, and authenticated by Mr. Anil Prakash, department of Biotechnology, Barkatullah university, Bhopal, M.P. where a voucher specimen No. has been submitted. (Voucher specimen No. 30).

Preparation of plant extract
Collected leaves of *Galinsoga parviflora* were converted into moderately coarse powder and extracted with solvent methanol for 40 hours by soxhlet. The solvent was removed under reduced pressure and the yield of extract was calculated.

Assessment of *in vitro* anti-arthritic activity
Inhibition of protein denaturation method: 10
The reaction mixture (0.5 ml) consisted of 0.45 ml bovine serum albumin (5% aqueous solution) and 0.05 ml of *Galinsoga parviflora* extract (100, 250 and 500 mcg/ml of final volume). pH was adjusted at 6.3 using a small amount of 1N HCl. The samples were incubated at 37 °C for 20 min and then heated at 57 °C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube.

Turbidity was measured spectrophotometrically at 660nm for control test 0.05 ml distilled water was used instead of extracts while product control test lacked bovine serum albumin. The percentage inhibition of protein denaturation was calculated as follows.

$$\text{Percent inhibition} = \frac{00\text{-}(\text{O.D. of test} - \text{O.D. of product control}) \times 100}{\text{O.D. of test}}$$

The control represents 100% protein denaturation. The results were compared with acetyl salicylic acid (250 mcg/ml) treated samples. The result were tabulated in Table 1.

Assessment of *in vitro* anti-arthritic Activity

The platelet rich plasma 0.13 X 10^7 for each assay was re-suspended in pH 7.4 tyrode buffer. The platelet aggregation was recorded as transmittance values of spectrophotometer measurement. To determine the *in-vitro* inhibition of platelet aggregation different concentrations of methanolic extract of *Galinsoga parviflora* like 100, 250 and 500 mcg/ml in DMSO (Dimethyl sulphoxide) were used. The platelet aggregation was induced with ADP at a concentration of 5 mcg/ml which is used as control. The Aspirin 200 mcg/ml is used as standard. The transmittance is recorded at interval of 1min for every 5 min. The result were tabulated in Table: 2 & Fig:1.

Statistical Analysis

Data are presented as the mean ± SEM of each triplicatetest. The analysis was performed by using Dunnett vs. Control test and by ANOVA. P‘0.05 were considered to be statistically significant.

Results and Discussion

Anti-arthritic activity

Anti-arthritic effect of methanolic extract of *Galinsoga parviflora* was studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of methanolic extract of *Galinsoga parviflora* on inhibition of protein denaturation is shown in table 1. Extract of *Galinsoga parviflora* at three different concentrations (dose levels) provided significant protection against denaturation of proteins. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in-vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. Obtained data stated that *Galinsoga parviflora* could be used as potent anti-arthritic agent.

Table 1: Effect of methanolic extract of *Galinsoga parviflora* on inhibition of protein denaturation

S. No.	Treatment	Protein denaturation (%)
1	*Galinsoga parviflora* (Linn.) (100 mcg/ml)	75.25 ± 3.25
2	*Galinsoga parviflora* (Linn.) (250 mcg/ml)	74.75 ± 2.75
3	*Galinsoga parviflora* (Linn.) (500 mcg/ml)	60.50 ± 3.55
4	Aspirin (200 mcg/ml)	45.50 ± 2.70

Anti-platelet activity

Platelet play an important role in the process of atherombosis by adhering to the damage region of endothelial surface. The activated platelets to platelet bond, bind also to leucocytes bring them in to a complex process of plague formation and growth. The anti-platelet therapy constitutes the best available tool for ameliorating the mechanism related to atherogenesis and have interestingly inhibited platelet aggregation. Platelets stick to the damaged vessel wall, they stick to each other (aggregate) and release ADP, thromboxane A_2 (TXA_2) which promote further aggregation, and thus a platelet plug is formed. In the veins, due to sluggish blood flow, the fibrinous tail is formed which traps RBC’s. Ati-platelet druds are more useful in arterial thrombosis, while anti-coagulants are more effective in venous thrombosis. The methanolic extract of *Galinsoga parviflora* showed significant anti-platelet activity at 500 mcg/ml.

Table 2: Percentage transmittance values of methanolic extract of *Galinsoga parviflora* for anti-platelet activity

S.N.	Concentration (mcg/ml)	0 min	1 min	2 min	3 min	4 min	5 min
1	ADP(5mcg/ml)	25.25	27.22	29.40	27.49	26.24	24.48
2	100	98.45	85.44	82.50	82.12	80.45	80.00
3	250	85.55	84.48	83.22	81.45	80.75	79.45
4	500	75.45	72.40	71.22	70.25	69.75	69.00
5	Aspirin (200mcg/ml)	90.45	89.75	85.45	82.00	81.25	80.55

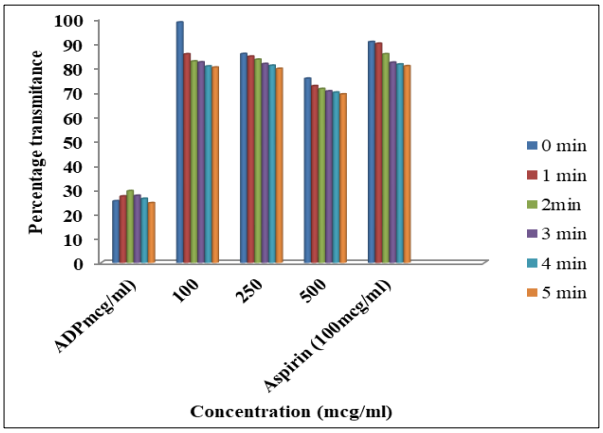

Fig 1: Effect of *Galinsoga parviflora* on anti-platelet activity

Conclusion

From the results obtained in the present study, it may be concluded that *Galinsoga parviflora* possess significant anti-arthritic as well as anti-platelet activity. Hence it could be beneficial for further work as active anti-arthritic agent and ant-iplatelet agent.

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