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Cytotoxic and Antimicrobial Activity of Common Invasive Alien Plant Species of Gopalganj, Bihar (India)

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Abstract In today's scenario, environment and lifestyle caused innumerable changes in human health. These changes are mostly negative in nature and have almost become irreversible; Diseases like cancer, diabetes, arthritis, rheumatoid arthritis, atherosclerosis and chronic inflammatory disorders have become very common. Taking into consideration the side effects and high cost of chemically synthesized agents, researchers are now turning towards traditional remedies and treatment. They turn out to be better medications with minimal side effects and less costs too. Therefore, some invasive alien species were selected which are common in Bihar region. These invading species have high biochemical activities, which help them spread and dominate in any regions. The purpose was to explore these biochemical activities of IAS (invasive alien species) to find better anti-inflammatory, anti-arthritic and antioxidant compounds from them. Five selected species were *Ageratum conyzoides* (L.) L, *Eupatorium adenophorum* Hort. Berol. ex Kunth, *Galinsoga parviflora* Cav. *Mikania micrantha* (L.) Willd. and *Parthenium hysterophorus* L. The extracts of the whole plant were tested on Cytotoxicity, anti-inflammatory, anti-arthritic and antioxidant activities. *Eupatorium adenophorum* Hort extract and *Parthenium hysterophorus* L. extract exhibit significant inhibitory action against Colon cell line (HT-29). *Mikania micrantha* (L.) extract was found to have highest antioxidant and anti-arthritic property with lowest IC50 of 32.23 μg/ml and 97.53 μg/ml respectively.

Keywords Antioxidant, Free Radical, Reactive Oxygen Species, Anti-arthritic, Anti-inflammatory, IC50, Invasive Alien Species

1. Introduction

Every ecosystem constitutes invasive alien species, animal, plant, insect and birds. Common terrestrial environment which faces this problem of invasive alien species is plants, mammals, and insects (Sharma *et al*., 2005). Alien species play integral role in field of farming and Forestry but turn out to be fatal for biodiversity when they tend to express capability to establish and dominate the native species (Sujay *et al.*, 2010).

The studies and literature have revealed that around 10% of the world's vascular plants (300,000) have the capability to invade other ecosystems and affect native biota in direct or indirect ways (Singh *et al*., 2006). About 18% of the Indian flora constitutes aliens, of which 55% are American, 30% Asian and Malaysian and 15% European and Central Asian species (Nayar, 1977). North-eastern U.P. shows higher prevalence of invading alien species as compared to entire Uttar Pradesh (Singh *et al*., 2010) and India (Reddy, 2008). A compilation of all the invasive alien plant species reveals that 103 invaders are common to the whole of the state of Uttar Pradesh (Singh *et al.*, 2010) and 95 species to the whole of India (Reddy, 2008). The invasive species which cover north-eastern Uttar Pradesh, around 70.5% are native to American continent. Other reports showed nearly

similar percentage share of plants native to tropical American. While Singh *et al*., 2010 reported 73% of invasive plant species of U.P., for Indian Himalayan region however, Sekar (2012) also noticed 73% invaders of American nativity. Reddy (2008) noticed 58% of the invasive flora of India to be natives of American continent. These alien species cover a very huge percentage of Bihar region and prove to be competitors to the native species. Properties like faster growth rate and more production of biomass as compared to native species, greater competitive capability, high reproductive efficiency with properties like large number of seeds, productive dispersal, vegetative reproduction, rapid establishment and other traits that help them adapt to new habitats (Simberloff *et al*., 2005 and Sharma *et al*., 2005). IAS also have potential to survive in extreme conditions and allelopathic in nature (Sharma *et al*., 2005 and Huang *et al*., 2009). Thus they could be a rich source of therapeutic properties which are still unexplored. Many plant derived products have been reported to show activity against leukemia cells, including resistant phenotypes (Senthilkumar *et al*., 2014). But invading alien species of Bihar is still far from completely explored. This work highlights majorly biological activities possessed by the extract reported in earlier work. Activities studied were cytotoxic activity, anti-arthritic activity, anti-inflammatory activity and antioxidant activity for *Ageratum conyzoides, Eupatorium adenophorum, Galinsoga parviflora, Mikania micrantha* and *Parthenium hysterophorus.*

2. Material and Methodology

Extraction

Plant samples were collected from various regions of Gopalganj, Bihar. Extraction was done by successive soxhlet extraction using solvents in increasing order of polarity *viz.* petroleum ether, ethyl acetate and methanol (Cowan, 1999). 25gm of each of the powdered plant was extracted successively with petroleum ether, ethyl acetate and methanol for 7hours. The filtrates obtained from soxhlet were evaporated to dryness in a rotary evaporator under reduced pressure. Dried powders of extracts were kept in sterile bottles at 4 ℃ in a refrigerator until used for analysis. The powdered samples were dissolved immediately before use in 10% DMSO to make four different concentrations of 12.5mg/ml, 25mg/ml, 50 mg/ml and 100mg/ml.

Bioactivity of the Extract

Cytotoxic Activity by MTT Assay

Viability and cell metabolic activity of Colon cell line (HT-29) were evaluated using MTT Assay (Mosmann, 1983). The method followed was the standard method of MTT Assay concisely described by first cells seeded in triplicate in microtitre plate (96 well plate) and incubated to grow and reach confluence. The media was then decanted and fresh media added to it. The cells were then left to grow in presence of plant extracts. After completion of incubation period (48hours), MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazoliu m bromide) (Sigma-Aldrich, India) reagent (5mg/ml) was added in each well except well with DMSO (indicating 100% cell viability) and left for 3.5 hours. Formazen crystals were dissolved in MTT solvent solution (Isopropanol+4mMHCl, 0.1% NP-40) and resultant was read on ELISA reader (HEALES Fully Automatic MB-580 Elisa Reader) at 560nm and subtracted from 670nm (background absorbance). Each fraction was tested at 4 different concentrations varying from 25mg/ml to 800 µg/ml. The % cell viability was determined graphically and calculated using the following formula:

$$50\ \%\ \text{cell viability} = (A_{570}\ \text{of treated cells} / A_{570}\ \text{of control cells}) \times 100)$$

Antioxidant Activity using DPPH Assay

DPPH Assay (Panda *et al*., 2011) is one of the most suitable methods to estimate the antioxidant activity of any product. DPPH is a stable organic free radical which absorbs at 517nm. During the reaction, it accepts an electron or a free radical species, it loses its purple colour. It is this purple color which causes absorption at 517nm (Paraskeva *et al*., 2008). The DPPH radical scavenging activity of acetone extracts was determined using the method described by Brand-William *et al*. (1995). 0.3 ml of different concentration (100, 200, 300, 400 and 500µg/ml) of sample was taken and made up to 0.4ml with distilled water. In this sample, added 0.6 ml of 100M DPPH reagent in methanol. The reaction mixture was incubated for 20minutes under dark and the reading was taken at 517nm. The decrease in absorbance at 517 nm was taken as the antioxidant capacity of the sample.

In-vitro anti-arthritic activity by Inhibition of Protein Denaturation Method (IPDM)

Anti-arthritic activity for plant extracts was assessed using protein denaturation method (Rajurkar *et al*., 2009). The method was performed using Bovine Serum Albumin (BSA). When Bovine Serum Albumin is heated, denaturation causes antigens to be expressed which are associated with type-III hypersensitivity reaction. These type III hypersensitivity reactions are associated with diseases like serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematous (Shravan *et al*., 2011). *In-vitro* anti-arthritic activity of the plant extracts done using Protein Denaturation Method of Sakat *et al.* (2010). The reaction mixture (5ml) included egg albumin (0.2 ml), phosphate buffered saline, 2.8ml (pH 6.4) and 2ml of Bwas orthobotrys The reaction mixture consisted of the 100µl test extracts (final concentration 9.77-1250µg/ml) and 100 µl of 5% aqueous solution of bovine serum albumin (BSA); pH was adjusted adding a small volume of glacial acetic acid. The sample extracts

were incubated at 37 ℃ for 20 min and then heated to 70 ℃ for 10 min. The mixture was allowed to cool for 10 min after which turbidity was measured at 660 nm. The blank comprised the sample and distilled water. Distilled water was used as the negative control. The positive control was diclofenac sodium (final concentration 0.61–78. Percentage inhibition was calculated using the formula:

% inhibition = 100*(Abs Sample - Blank/control - 1)

The IC_{50} was calculated from a graph of inhibition against the different concentrations. The experiment was carried out in triplicate.

In-Vitro Anti Inflammatory Assay of membrane stabilisation

The effects of the plant extract on haemolysis of HRBC induced by heat and distilled water was evaluated using the method of Shinde et al. (1999) with some modifications. Different concentrations of the sample (100, 200, 300, 400 and 500 ug/ml) was prepared by diluting the samples in 1% acetic acid solution. Reaction mixture was prepared containing 1 ml of the phosphate buffer, 2 ml of Hypo saline, 0.5ml of HRBC Suspension and 1 ml of sample. The sample mixture was incubated at 37 ℃ for 30 minutes. The tubes were allowed to cool down after the end of incubation period. Centrifuged the reaction mixture at 3000 rpm for 5 minutes and supernatant was decanted and the content of haemoglobin was measured by reading the absorbance at 560 nm. Diclofenac Sodium was used as the standard. Percent Protection was calculated.

3. Result and Discussion

The extracts of *Ageratum conyzoides* (ACE), *Eupatorium adenophorum* (EAE), *Galinsoga parviflora*, *Mikania micrantha* and *Parthenium hysterophorus* (PHE) were analyzed for bioactivity by determining their cytotoxic activity, anti-Inflammatory activity, anti-arthritic activity and antioxidant activity.

Cytotoxic Activity by MTT

The results showed that *Eupatorium adenophorum* (EAE) and *Parthenium hysterophorus* (PHE) extracts exhibit significant inhibitory action against colon cell line (HT-29) with lowest IC50 concentrations (3.403µg/ml and 4.887µg/ml) needed to kill 50% of the targeted cells. The least significant activity was observed for *Mikania micrantha* extract (MME) against HT-29, with IC50 at 8.700µg/ml, followed by *Galinsoga parviflora* extract (PHE) at IC50-7.290µg/ml and *Ageratum conyzoides* extract (ACE) IC50-6.740µg/ml. Activities of all the extracts are summarized in Table 1 and comparative cytotoxic activity of the extracts are shown in Figure 1.

Table 1. Cytotoxicity Assay IC50 concentration of Plant extracts against Colon cell line (HT-29)

S.No.	Plant extract	Sample Code	IC50 (µg/ml) ±S.D.*
1	*Ageratum conyzoides* (L.) L.	ACE	6.740 ±0.185
2	*Parthenium hysterophorus*L.	PHE	4.887 ±0.822
3	*Eupatorium adenophorum* Hort.Berol. ex Kunth	EAE	3.403 ±0.239
4	*Galinsoga parviflora*Cav.	GPE	7.290 ±0.445
5	*Mikania micrantha* (L.) Willd.	MME	8.700 ±0.334
*S.D. – Standard deviation			

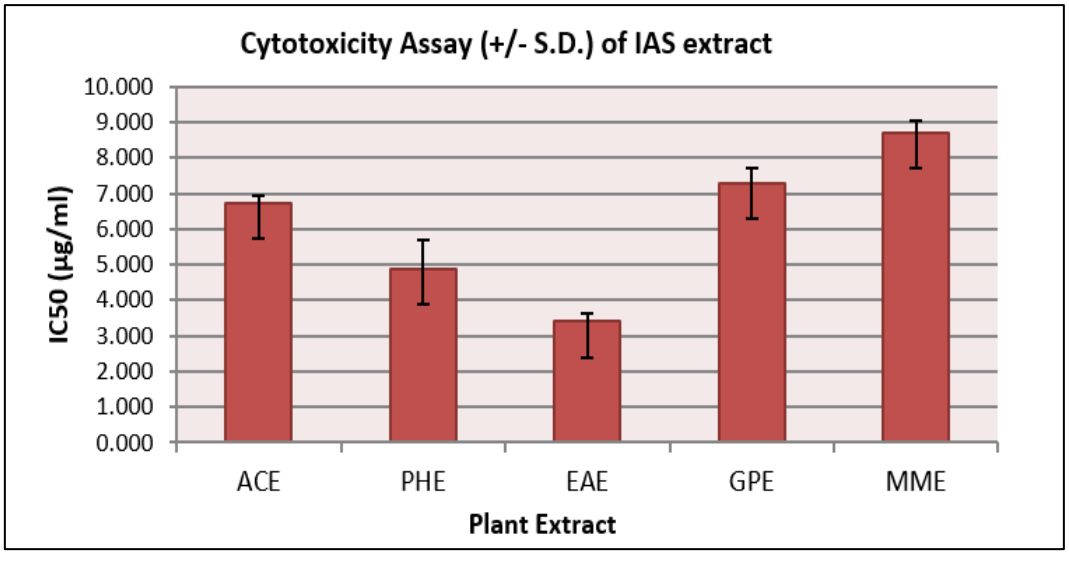

Figure 1. Comparative IC50 concentration of Plant extracts against Colon cell line (HT-29)

Antioxidant Activity using DPPH Assay

In this study, the extracts showed variable range of antioxidant property, causing the mixture to change its colour. The antioxidant property was seen to increase as the concentration was increasing. Thus a dose dependent process was recorded.

The concentration range helped to determine IC50 in each plant species extract. The IC50 values ranged from 32.23 µg/ml- 207.27 µg/ml (as shown in Table 2 and Figure 2). As, being inversely related, the lower the IC_{50} value, the higher is the antioxidant activity possessed by tested compound (Liu *et al.*, 2009). Therefore, *Mikania micrantha* (MME) was found to have the highest antioxidant property with the lowest IC50 of 32.23 µg/ml followed by *Parthenium hysterophorus* (53.36 µg/ml) and *Eupatorium adenophorum* (55.81 µg/ml). *Ageratum conyzoides* extract (ACE) had low antioxidant property with very high concentration (127.49 µg/ml) required for activity. *Galinsoga parviflora* extract (GPE) had shown least antioxidant with IC50 of 207.27 µg/ml. Ascorbic acid is used as standard for DPPH Assay, its known significant antioxidant activity helps to compare the activity of the plant extracts. The IC50 for ascorbic acid was observed to be 12.42µg/ml which is quite, thus *Mikania micrantha* extract (MME) showed comparable results.

Table 2. IC50 concentration of Plant extracts for Antioxidant Assay

S.No.	Plant extract	Sample Code	IC50 (µg/ml) ±S.D. *
1	*Ageratum conyzoides* (L.) L.	ACE	127.49 ±4.833
2	*Parthenium hysterophorus* L.	PHE	53.36 ±4.413
3	*Eupatorium adenophorum* Hort.Berol. ex Kunth	EAE	55.81 ±1.680
4	*Galinsoga parviflora* Cav.	GPE	207.27 ±38.439
5	*Mikania micrantha* (L.) Willd.	MME	32.23 ±7.817
*S.D. – Standard deviation			

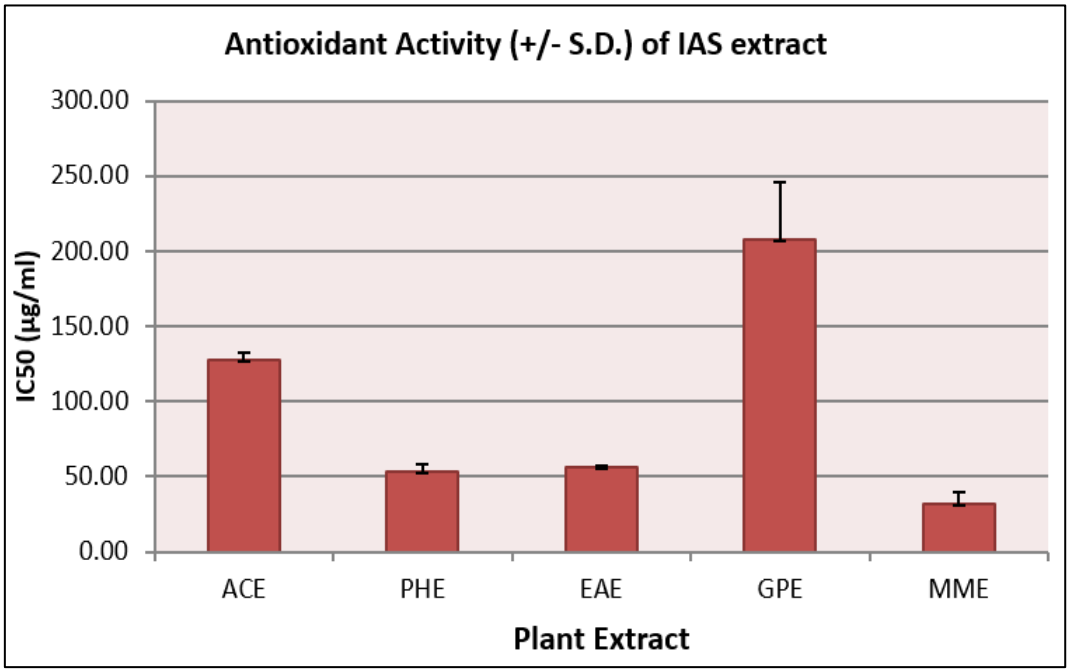

Figure 2. Comparative Antioxidant activity (IC50) of five alien species extract incubated for 30 minute with DPPH (0.1mM) at 517 nm as compared to standard Ascorbic acid.

In-vitro anti-arthritic activity by Inhibition of Protein Denaturation Method

The extracts showed dose-dependent response in the vitro anti-arthritic test. The IC_{50} concentrations of the plant extract ranged from 97.53µg/ml-129.91 µg/ml. The IC50 concentration (Table 3 and Figure 3) shows that the extracts do not show very significant anti- arthritic activity.

Diclofenac sodium used as standard showed IC50 concentration to be 47µg/ml. Thus *Mikania micrantha* (MME) showed IC50 97.53µg/ml, which was closest to Diclofenac sodium. In rheumatoid arthritis, protein denaturation takes place and auto antigen production is caused as a result of protein denaturation. This protein denaturation may involve effect on interactions like hydrogen bonding, electrostatic, hydrophobic and disulphide bonds Arya *et al*. (2014). Other extracts showed much higher IC50 concentrations as compared to Diclofenac sodium. IC50 of *Ageratum conyzoides* (ACE)-104.42 µg/ml, *Parthenium hysterophorus*. extract (PHE)-116.15 µg/ml, *Eupatorium adenophorum* Hort extract (EAE)-112.38 µg/ml and *Galinsoga parviflora* extract (GPE)- 129.91 µg/ml.

Table 3. IC50 concentration of Plant extracts for In-vitro Anti-Arthritic Assay

S.No.	Plant extract	Sample Code	IC50 (µg/ml) ±S.D.*
1	*Ageratum conyzoides* (L.) L.	ACE	104.42 ±0.477
2	*Parthenium hysterophorus* L.	PHE	116.15 ±1.519
3	*Eupatorium adenophorum* Hort.Berol.ex Kunth.	EAE	112.38 ±0.454
4	*Galinsoga parviflora* Cav.	GPE	129.91 ±1.906
5	*Mikania micrantha* (L.) Willd.	MME	97.53 ±0.849

*S.D. – Standard deviation

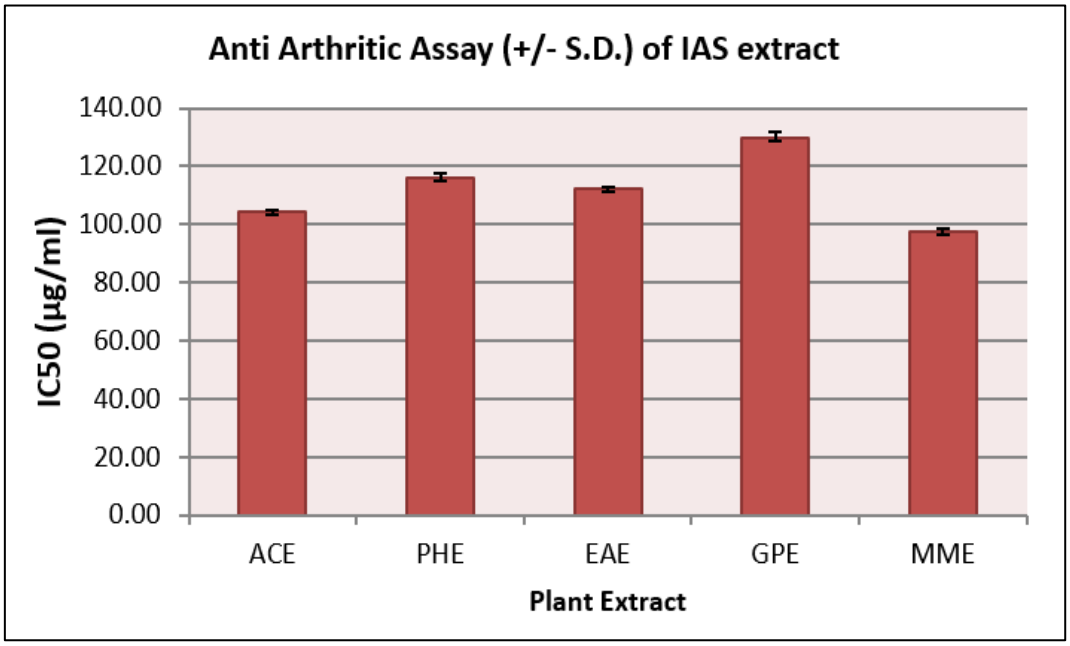

Figure 3. Comparative IC50 concentration of Plant extracts for In-vitro Anti Arthritic Assay

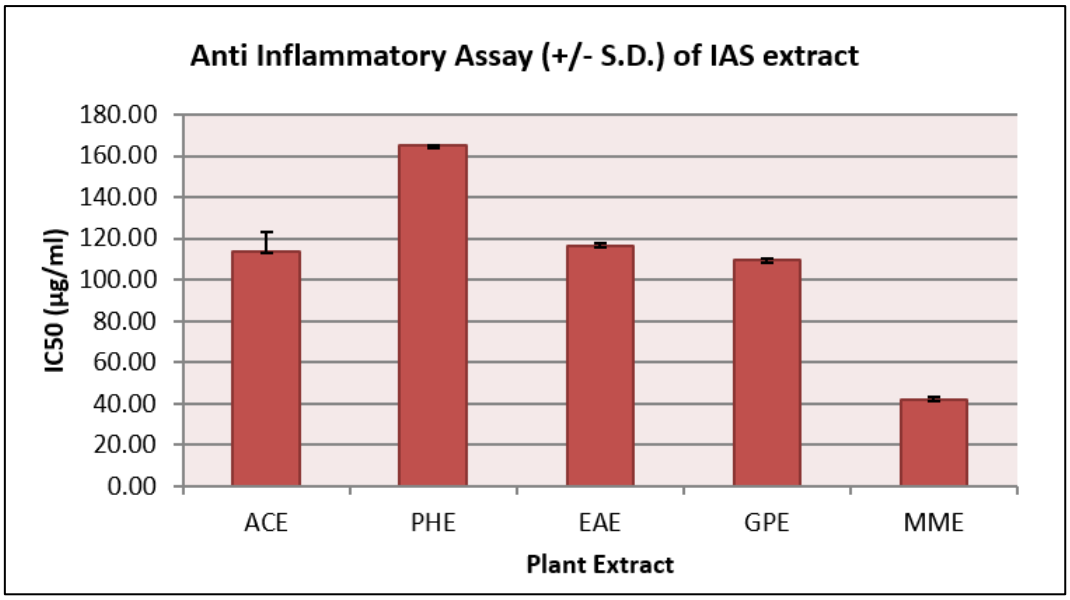

Figure 4. Comparative IC50 concentration of Plant extracts for In-vitro Anti Inflammatory Assay

In-vitro Anti-Inflammatory Assay of membrane stabilization

An efficient method for anti-inflammatory activity for any agent is determined by observing protective effect on heat and hypotonic saline induced erythrocyte lysis. We aim to find some new anti-inflammatory agents using natural source like plant, which show their potency with lesser side effects. Thus they prove to be better substitutes for harmful chemical agents used as anti-inflammatory compounds.

Table 4. IC50 concentration of Plant extracts for In-vitro Anti Inflammatory Assay

S.No.	Plant extract	Sample Code	IC50 (µg/ml) ±S.D. *
1	*Ageratum conyzoides* (L.) L.	ACE	113.90 ±9.492
2	*Parthenium hysterophorus* L.	PHE	165.14 ±0.157
3	*Eupatorium adenophorum* Hort.Berol. ex Kunth	EAE	116.78 ±1.279
4	*Galinsoga parviflora* Cav.	GPE	109.55 ±0.533
5	*Mikania micrantha* (L.) Willd.	MME	42.14 ±1.362
*S.D. – Standard deviation			

The *Mikania micrantha* whole plant extract showed comparable significant activity with the common standard diclofenac sodium (Rajalakshmi and Harindran, 2013; Arya and Patni, 2013). All the plant extracts showed significant anti-inflammatory activity (as shown in Table 4 and Figure 4) which could be due to their phytochemical profile such as alkaloids, flavonoids, tri-terpenoids and phenols, glycosides etc.

4. Conclusion

These free radical species are extremely reactive causing harm to human health, if it reaches to certain extent. Besides being natural process, free radical species generation also takes place due to some human activities and lifestyle. Major causes are smoking, excessive exposure to pollution, pesticides and radiations of environment. Few major diseases which are caused due to predisposition of oxidative stress are cancer, diabetes, arthritis, rheumatoid arthritis, atherosclerosis and chronic inflammatory disorders. We focus on some natural sources for bioactive components which could help to fight these problems. Thus, it is concluded that these plants possess good anti-inflammatory and antioxidant activities which could prove beneficial after extensive research and better product development in future.

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