

## Screening of Stem Extracts of *Andrographis echinoides* for Anti-Inflammatory, Anti-Arthritic, Anti-Microbial, and Anti-Cancer Activity against MCF-7 Human Breast Cancer Cell Lines

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Nowadays breast cancer in rheumatoid arthritis patients is the most controversial one. Rheumatoid arthritis is an inflammatory autoimmune disorder. RA may induce the order disease like cancer especially breast cancer in most women. Breast cancer starts when the breast cell begins to grow out of control. Inflammatory, anticancer drugs may show severe side effects. To overcome these herbal plants are used to make drastic changes in medical treatments. The main objective of this paper is assessment of Anti-inflammatory, anti-arthritic, anti-microbial and anti-cancer activity against the Michigan cancer foundation (MCF-7) human breast cancer cell line using the extracts *Andrographis echinoides*. Anti-inflammatory and anti-arthritic activity show maximum inhibition of albumin denaturation, anti-proteinase, hypo-tonicity induced hemolysis, anti-lipoxygenase activity with standard drugs such as aspirin, diclofenac sodium and indomethacin at the concentration of 100µg/ml under in vitro condition. Thus the potential inhibition of protein (egg albumin) denaturation (85.80%) and for the human red blood cell (HRBC) membrane stabilization model 85.50% was observed at the concentration of 100µg/ml. The antioxidant and cytotoxicity activity of *Andrographis echinoides* showed better inhibition in a dose-dependent manner against MCF-7 human breast cancer cell lines. The antimicrobial activity of *Andrographis echinoides* showed maximum inhibition of *E.coli* (11mm), *Enterococcus aerogenes* and *Staphylococcus aureus* (10mm), and *Candida albicans* (11mm) at 100µg/ml concentration. The innovation of the present study revealed that the stem of *Andrographis echinoides* have potential source for natural anti-arthritic, cytotoxicity, anti-microbial, antioxidant activity, anti-inflammatory and anticancer activities.

**Keywords:** Aspirin; Breast cancer Michigan Cancer Foundation (MCF-7); Hemolysis; Inflammation; Rheumatoid arthritis.

Rheumatoid arthritis is a persistent, inflammatory autoimmune illness which affects the joints. It also affects the tissue which surrounds the joints<sup>1</sup>. Many mediators like TNF- $\alpha$ , IL-2 and enzymes are involved in rheumatoid arthritis<sup>2, 3, 4</sup>. Currently, a combination of many diseases may develop during the RA disease. And one of the

fair problems is cancer, especially breast cancer in most females<sup>5</sup>. Irregular host immune response is examined as a source for the development of a tumor. Due to the immune suppression is the increased chance of cancer<sup>6, 7</sup>. Current studies exhibited that the promising threat of breast cancer in RA, however, the occurrence of breast

cancer in the population stays doubtful. Cancer is a proliferating disease combining physical, environmental, metabolic, chemical, and genetic factors<sup>8</sup>. In the US country, women Breast cancer is the major cause of death and the cell targeting drug development without affecting normal cells is a challenging task in the field of drug discovery<sup>9</sup>. Cytotoxic agents are expensive and induce severe side effects<sup>10,11</sup>. Several medicinal plant species and their phytochemicals inhibit the progression and development of cancer.

*Andrographis echinoides* come under the family of Acanthaceae (GopuramThangi). It is an ayurvedic herb plant used in the treatment of many ailments such as anti-inflammatory, anti-arthritic, antimicrobial, anti-ulcer, antioxidant activity, hair problems, etc., Their phytochemical constituents like flavonoids, tannins, phenol, glycosides, terpenoids, saponins, steroids, etc., *Andrographis echinoides* is a medicinally valuable species widely distributed in the tropical region in South Asian countries. This paper reveals that anti-inflammatory, anti-arthritic, anti-microbial and anticancer activity against breast cancer cell line (MCF-7) from *Andrographis echinoides* stem extract.

## MATERIALS AND METHODS

### Plant collection

*Andrographis echinoides* stem was procured and authenticated by botanist, Research associate, CARISM and SRC SASTRA Deemed to be University, Tirumalaisamuthiram, Thanjavur (Voucher no. SRC SASTRA 0005)

### Preparation of ethanolic extract

The stem of *Andrographis echinoides* was rinsed with running water, slice into small pieces and shade dried at 35-40C for a week, after that make it into a uniform powder and subjected for soxhlet extraction process by using ethanol as solvent<sup>12</sup>.

Methodology for the anti-inflammatory activity of ethanolic extract of *Andrographis echinoides*

### Inhibition of albumin denaturation

5ml of reaction mixture contains 0.2ml of egg albumin, different concentration of extract (20-100µg/ml) and 2.8ml of phosphate buffered saline. Double distilled water served as a control.

Then incubated at 37C for 15mins and heated at 70C for 5mins. Cooled it and the absorbance was observed at 660nm. Diclofenac sodium was taken as standard<sup>13, 14</sup>. The protein denaturation inhibition in percentage was calculated as below

$$\text{Percentage of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

### Anti-proteinase action

2ml of reaction mixture contains 1ml trypsin, 1ml TrisHCl buffer (pH 7.4) and 1ml of different concentration of extract (20-100µg/ml). Then incubated at 37C for 5mins then add 1ml of casein and again incubated for 20mins. 1ml of perchloric acid was added to stop the reaction. The dark suspension was centrifuged and the supernatant absorbance was measured at 210nm<sup>15</sup>. The proteinase inhibitory activity in percentage was calculated.

$$\text{Percentage of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

### Hypotonicity induced hemolysis

Different concentrations of extracts (20-100µg/ml) were taken. Then, 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension of mixed solution served as control. Standard as a diclofenac sodium. Absorbance read at 560nm<sup>16</sup>. The hemolysis percentage was evaluated by taking hemolysis produced in the control as 100%

$$\text{Percentage of protection} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

### Anti-lipoxygenase activity

Different concentrations of extracts (20-100µg/ml) were dissolved in 0.25ml of 2M borate buffer pH 9 and then 0.25ml of lipoxygenase enzyme solution was added and incubated at 25C for 5mins. Then 1ml of linoleic acid solution was added and mixed well then absorbance was read at 234nm. Standard used as Indomethacin<sup>17</sup>. The percent inhibition was calculated from the following equation,

$$\text{Percentage of inhibition} = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 100$$

For IC<sub>50</sub> value, dose response curve was plotted, in which 50% of maximum scavenging capacity was calculated. All tests were performed in triplicate and the average values are taken.

**Evaluation of anti-arthritic activity****Inhibition of protein denaturation model**

A 5ml reaction mixture containing 0.2ml of egg albumin, 2.8ml of phosphate buffer and 2ml of distilled water and various concentrations of extract were taken. Diclofenac sodium as the standard drug and the procedure was performed as the same<sup>18, 19</sup>. The samples were incubated for 15mins at 37C and heated at 70C for 5 mins. After cooling, the absorbance is measured using a UV-Visible spectrophotometer at 660nm. The protein denaturation inhibition in percentage was calculated using the following formula,

$$\text{Percentage of inhibition} = (\text{Control} - \text{Test} / \text{Control}) \times 100$$

**Human red blood cell (HRBC) membrane stabilization model****Assay of membrane stabilizing activity**

Different concentrations of extracts, reference sample and control were taken, then mixed with 1ml of phosphate buffer, 2ml of hyposaline and 0.5ml of HRBC suspension. All the mixtures were incubated for 30mins at 37C. The liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560nm. The hemolysis percentage was evaluated by taking the hemolysis produced in control as 100%<sup>20, 21</sup>. The percentage of HRBC membrane stabilization or protection was observed as follows,

$$\text{Percentage of inhibition} = (\text{Control} - \text{Test} / \text{Control}) \times 100$$

**Antioxidant activity of *Andrographis echinoides* by DPPH assay**

Different concentrations of ethanolic extracts of *Andrographis echinoides* were taken. 500µl of DPPH enzymes was added to all the test tubes. The mixture was allowed to stand for 5mins and observed at 517nm. The absorbance zero was set with ethanol. Ethanol and DPPH solution mixture was taken as blank. This experiment was performed in a triplicate manner<sup>22</sup>. DPPH activity of the tested samples, reveals as a percentage of inhibition which were calculated as follows,

$$\text{Percentage of inhibition DPPH activity} = (\text{Control} - \text{Test} / \text{Control}) \times 100$$

Test and blank sample marked as A and B. The graph was plotted as percent inhibition vs concentration of the sample. 50% of inhibition activity was measured for each solution.

***In vitro* anticancer studies of ethanolic extract of stem of *Andrographis echinoides*****MTT assay**

*In vitro* cytotoxicity was carried out using *Andrographis echinoides*. The sample used for this activity is MCF-7 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. By trypsinization, a MCF-7 cell line of 15ml was harvested. After, the harvested cells were plated in a 96-well tissue of DMEM (10% FBS and 1% antibiotic solution) culture plate with a density of  $1 \times 10^5$  cells/well (200µl) and incubated at 37C for 24-48hrs. Then, the wells rinsed with PBS solution and handled with different concentrations of the herbal extract in non-serum DMEM medium. It was performed in a triplicate manner. By the continuation of the incubation period, MTT (20µl of 5mg/ml) was taken in each plate and the cells were incubated for next 2-4hrs for the formation of purple precipitates were viewed under an inverted microscope. Finally, the cells were washed with a PBS solution (200µl). The formazan crystals were dissolved, DMSO (100µl) was taken and the plate was shaken for 5mins. Each well absorbance was observed at 570nm using a microplate reader (Thermo Fisher Scientific, USA), and the percentage of viability of cell and IC<sub>50</sub> value was calculated by Graph Pad Prism 6.0 software (USA)<sup>23</sup>.

**Collection of test organisms**

The antimicrobial activity of *Andrographis echinoides*, was carried out using three strains [*Escherichia coli* (MTCC 25922), *Enterococcus aerogenes*, *Staphylococcus aureus* (MTCC 27853)] were prepared as test organisms. The fungal test sample *Candida albicans* (MTCC 282) in clinical trials was taken. The microorganisms were collected from Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

**Antibacterial activity of ethanolic extract of *Andrographis echinoides* (Disc diffusion method)**

Antibacterial activity was performed using ethanolic extract of *Andrographis echinoides* by disc diffusion method. Petri dishes (60mm) were taken and Muller Hinton Agar was inoculated with organisms. 10µl of ethanolic extracts of 20-100µg/ml was poured in a sterile disc. Then, the discs were placed on the agar plates and kept at room temperature for 30mins due to compound diffusion.

Respective solvent used as control. Finally, sterile petri dishes were incubated for 24hrs at 37C and the inhibitory zone was measured in millimeters and the experiment was repeated twice<sup>24</sup>.

#### Determination of the antifungal activity of ethanolic extract of *Andrographis echinoides* by disc diffusion method

The ethanolic extracts of *Andrographis echinoides* were determined using the disc diffusion method. The petri dishes (60mm) were poured with Sabouraud's dextrose agar (SDA) and inoculated with test organisms. 10µl of ethanolic extracts of 20-100µg/ml was poured in a sterile disc. Then, the discs were placed on the agar plates and kept at room temperature for 30mins due to compound diffusion. Respective solvent used as control. Finally, sterile petri dishes were incubated for 72hrs. The inhibitory zone was measured in millimeters and the experiment was repeated twice<sup>25</sup>.

## RESULT AND DISCUSSION

The phytochemical analysis of ethanolic extract of *Andrographis echinoides* indicated the occurrence of anthocyanins, saponins, flavonoids, tannin, steroids, cardiac glycosides, leuco anthocyanin, coumarin, glycosides, terpenoids, emodin, phenol, alkaloids, proteins and absence of anthraquinone, carbohydrates and phlorotannins. The tannin has highest quantity followed by alkaloids (0.163mg/g), saponins (0.007mg/g), flavonoids (0.015mg/g), phenol (0.046mg/g), tannin (0.055mg/g) and terpenoids (0.004mg/g) respectively.

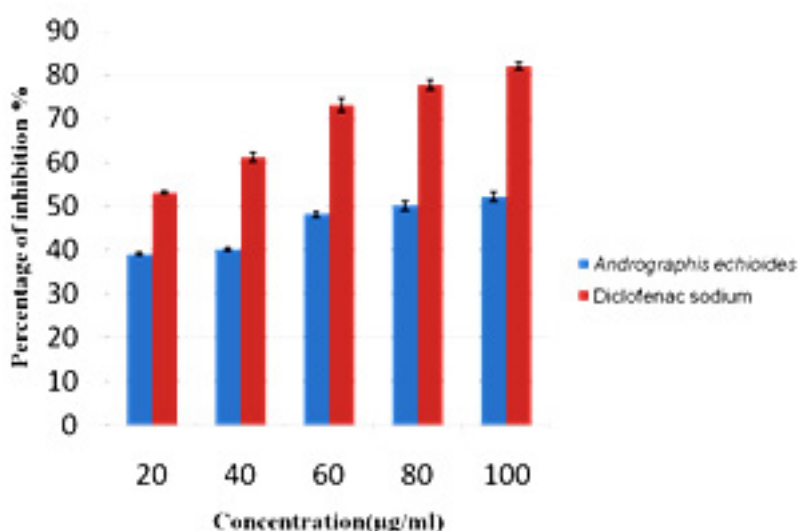
#### *In-vitro* anti-inflammatory activity

##### Inhibition of albumin denaturation

Normally, Denaturation of protein describes the losses of secondary and tertiary structure of protein and also for biological protein

**Table 1.** Albumin denaturation inhibition of *Andrographis echinoides* extracts with standard drug Diclofenac sodium

Concentration(µg/ml)	% of albumin denaturation	
	<i>Andrographis echinoides</i>	Diclofenac Sodium
20 (µg/ml)	39.13±0.34	53.16±0.22
40 (µg/ml)	40.18±0.45	61.25±1.03
60 (µg/ml)	48.24±0.67	73.10±1.56
80 (µg/ml)	50.16±1.09	77.69±1.16
100 (µg/ml)	52.21±0.65	82.00±0.87



**Fig. 1.** Inhibition of albumin denaturation

because of external intensity or compounds as strong base and acid, a solvent etc., In a previous study, some of the plant extracts that have the ability to block the denaturation mechanism in proteins were investigated under anti-inflammatory activity<sup>27</sup>. From this study, *Andrographis* is effective and has the ability for the inhibition of denaturation of albumin as shown in figure 1. 52% of potential inhibition of *Andrographis* was

determined at 100µg/ml. The standard drug of Diclofenac sodium inhibits up to 82% with 100µg/ml by comparison with control as predicted in Table 1

**Anti-proteinase activity**

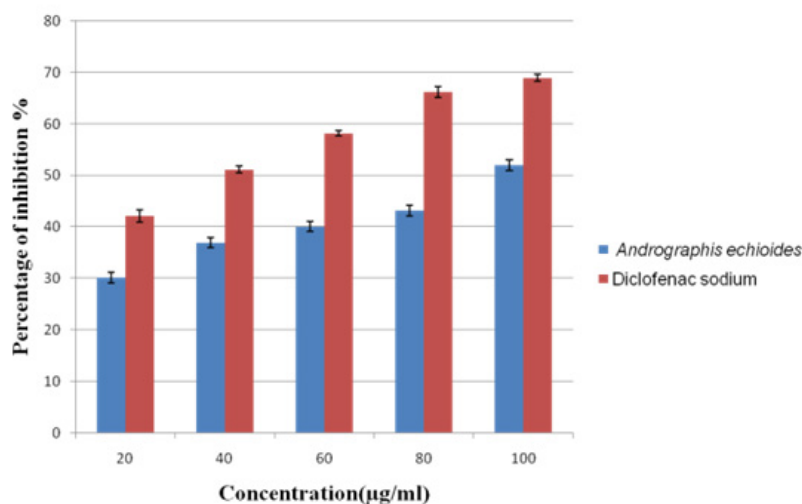
Basically, neutrophils are the origin of serine protease and are present in lysosomes. In a previous study, the authors reported that the proteinase enzyme of leucocytes has the ability to

**Table 2.** Anti proteinase activity of *Andrographis echiodides* extracts vs Diclofenac sodium

Concentration(µg/ml)	% of Anti proteinase activity	
	<i>Andrographis echiodides</i>	Diclofenac sodium
20 (µg/ml)	30.17±0.26	42.11±1.22
40 (µg/ml)	37.45±0.28	51.09±0.67
60 (µg/ml)	40.34±0.37	58.21±0.52
80 (µg/ml)	43.90±1.09	66.18±1.09
100 (µg/ml)	52.23±0.39	69.00±0.65

**Table 3.** Hypotonicity activity of *Andrographis echiodides* extracts with standard drug diclofenac sodium

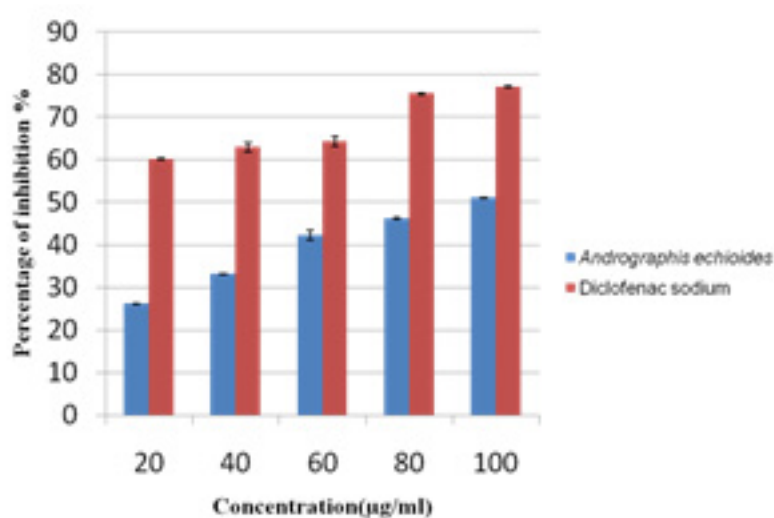
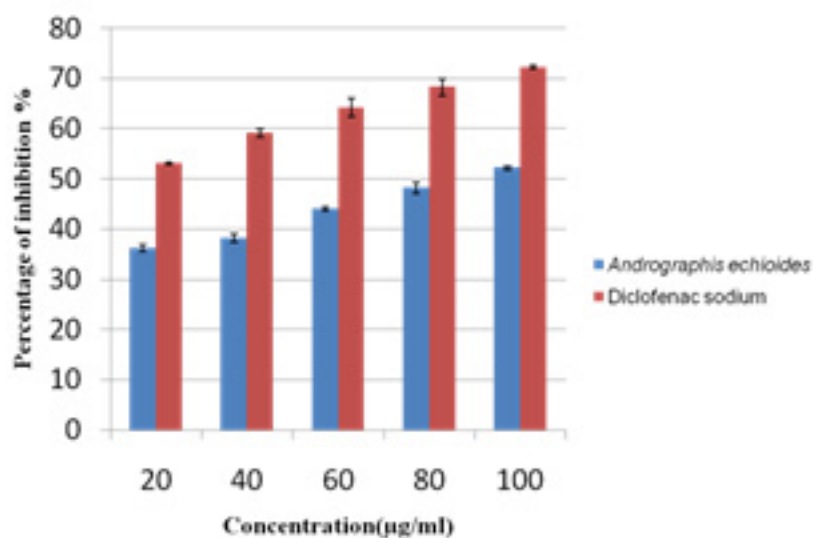
Concentration(µg/ml)	% of Hypotonicity induced haemolysis	
	<i>Andrographis echiodides</i>	Diclofenac sodium
20 (µg/ml)	26.13±0.15	60.12±0.12
40 (µg/ml)	33.09±0.18	62.91±1.07
60 (µg/ml)	42.17±1.09	64.22±1.15
80 (µg/ml)	46.21±0.20	75.43±0.16
100 (µg/ml)	51.00±0.15	77.08±0.12



**Fig. 2.** Anti proteinase activity

**Table 4.** Anti-lipoxygenase activity of ethanolic extract of *Andrographis echioides* with standard drug diclofenac sodium

Concentration( $\mu\text{g/ml}$ )	% Anti lipoxygenase activity	
	<i>Andrographis echioides</i>	Diclofenac sodium
20 ( $\mu\text{g/ml}$ )	36.16 $\pm$ 0.71	53.00 $\pm$ 0.21
40 ( $\mu\text{g/ml}$ )	38.19 $\pm$ 0.90	59.12 $\pm$ 0.82
60 ( $\mu\text{g/ml}$ )	44.00 $\pm$ 0.48	64.17 $\pm$ 1.90
80 ( $\mu\text{g/ml}$ )	48.21 $\pm$ 1.09	68.23 $\pm$ 1.65
100 ( $\mu\text{g/ml}$ )	52.16 $\pm$ 0.32	72.15 $\pm$ 0.32

**Fig. 3.** Hypotonicity activity**Fig. 4.** Anti-lipoxygenase activity

damage the tissues of inflammatory actions and an important level of protection was developed for enzyme inhibitors. Different concentrations of proteinase inhibition activity were predicted in table 2. *Andrographis* showed potential inhibition of 52% at 100µg/ml and diclofenac sodium inhibits maximum of 69% at 100µg/ml as shown in figure 2.

**Hypotonicity-induced haemolysis**

From this study, the result observed as different concentrations (20 to 100µg/ml) of *Andrographis* prevents the RBC membrane lysis by the solution of hypotonic as shown in table 3. At the concentration of 100µg/ml 51% of potential inhibition of *Andrographis* and 77% of inhibition of Diclofenac sodium was observed as shown in figure 3.

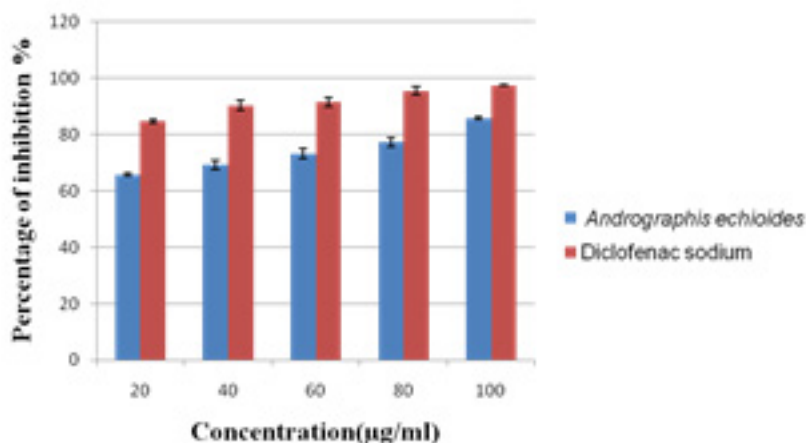
**Table 5.** Anti-arthritic activity of *Andrographis echiodes* with standard drug diclofenac sodium by protein denaturation method

Concentrations (µg/ml)	Protein denaturation (%)	
	<i>Andrographis echiodes</i>	Diclofenac sodium
20 (µg/ml)	65.79±0.36	84.65±0.55
40 (µg/ml)	69.31±1.66	90.35±1.79
60 (µg/ml)	73.13±1.78	91.49±1.64
80 (µg/ml)	77.35±1.54	95.53±1.59
100 (µg/ml)	85.80±0.38	97.43±0.28

Each values was obtained by calculating the average of three experiments and data are presented as mean ± SEM

**Table 6.** Anti-arthritic activity of *Andrographis echiodes* with standard drug diclofenacsodium using HRBC membrane stabilization method

Concentrations(µg/ml)	HRBC membrane stabilization assay (%)	
	<i>Andrographis echiodes</i>	Diclofenac sodium
20 (µg/ml)	66.71±0.89	69.66±0.38
40 (µg/ml)	70.00±1.69	72.42±1.25
60 (µg/ml)	74.30±1.81	77.19±1.23
80 (µg/ml)	82.59±1.83	85.95±1.47
100 (µg/ml)	85.50±0.49	86.12±0.29



**Fig. 5.** Protein denaturation

**Anti-lipoxygenase activity**

In screening methods, a medicinal plant contributes a great mechanism in the inhibition of lipoxygenase enzyme activity. In this experiment, *Andrographis* showed inhibition in a dose-

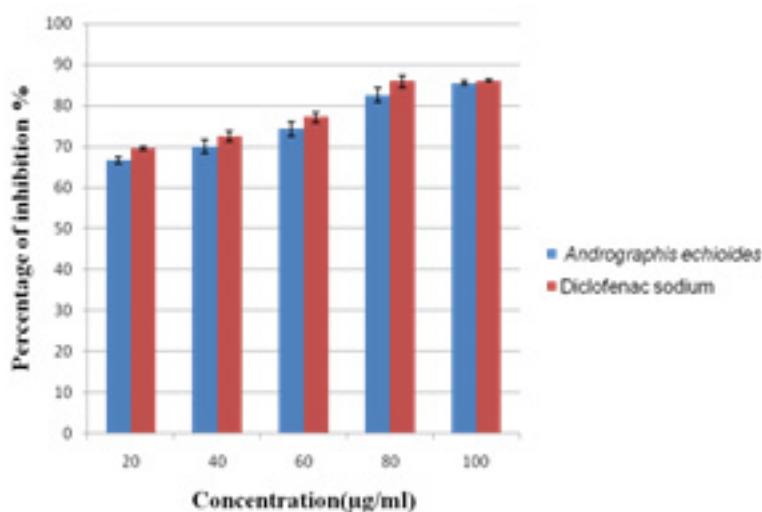
dependent manner as predicted in table 4. As per the result, a well inhibition was observed at 100µg/ml of 52% of *Andrographis* and 72% of Diclofenac as observed in figure 4. It showed better anti-lipoxygenase activity.

**Table 7.** Antioxidant activity of ethanolic extract of stem of *Andrographis echinoides* by DPPH activity

Concentration(µg/ml)	<i>Andrographis echinoides</i>	Ascorbic acid
20µg/ml	66.93±0.41	71.07±1.14
40µg/ml	67.74±0.60	73.43±1.05
60µg/ml	70.16±0.37	76.15±1.34
80µg/ml	80.64±0.24	82.32±1.91
100µg/ml	82.25±0.22	85.03±1.45

**Table 8.** Cell viability (%) test

Tested sample concentration (µg/ml)	Cell viability (%) (in triplicates)			Mean value (%)
Control	100	100	100	100
100 µg/ml	34.91	30.75	25.07	28.25
90 µg/ml	45.20	52.20	55.26	48.89
80 µg/ml	54.61	59.36	51.20	53.56
70 µg/ml	62.48	63.58	62.05	60.71
60 µg/ml	68.17	66.86	55.70	61.58
50 µg/ml	71.02	75.83	76.14	63.67
40 µg/ml	77.36	69.05	83.49	65.64
30 µg/ml	87.43	88.96	94.87	94.43
20 µg/ml	89.40	95.09	89.84	96.45
10 µg/ml	112.59	100.87	91.90	99.45



**Fig. 6.** Membrane stabilization of HRBC



### Evaluation of anti-arthritic activity

#### Protein denaturation inhibition

In this experiment both *Andrographis* (65% to 85%) and Diclofenac drug (84% to 97%) showed potential inhibition in a dose-dependent manner (20, 40, 60, 80 and 100 µg/ml) as shown in table 5. The denaturation of protein inhibition was well observed as shown in figure 5. A

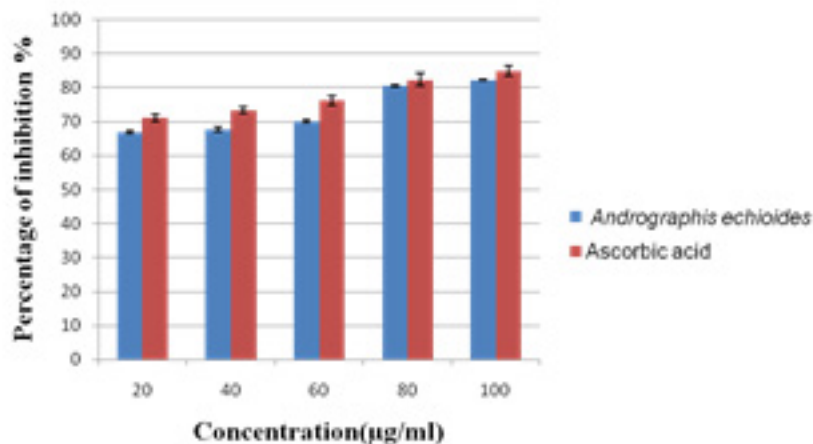
previous study suggested that the inhibition of protein denaturation was analyzed for the study of Ashwagandha, *Mikaniascandens* and Coffee<sup>26,27,28</sup>.

#### Membrane stabilization mechanism of Human red blood cell (HRBC)

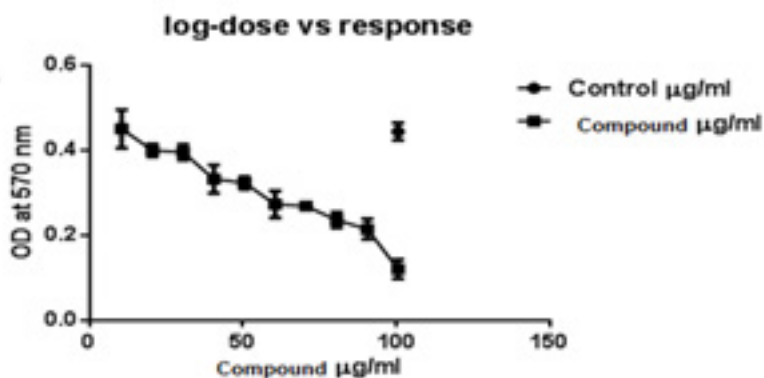
The standard drug Diclofenac sodium of various concentrations (20, 40, 60, 80 and 100 µg/ml) exhibited stabilization towards the

**Table 9.** Antibacterial activity of ethanolic extract of *Andrographis echinoides*

Plant extracts	Concentrations (µg/ml)	<i>Escherichia Coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Enterococcus aerogenes</i> (mm)
Extracts	20µg/ml	0	0	0
	40µg/ml	0	0	0
	60µg/ml	8	0	0
	80µg/ml	10	9	9
	100µg/ml	11	10	10
Ethanol	10 µl/disc	0	0	0



**Fig. 7.** Antioxidant activity



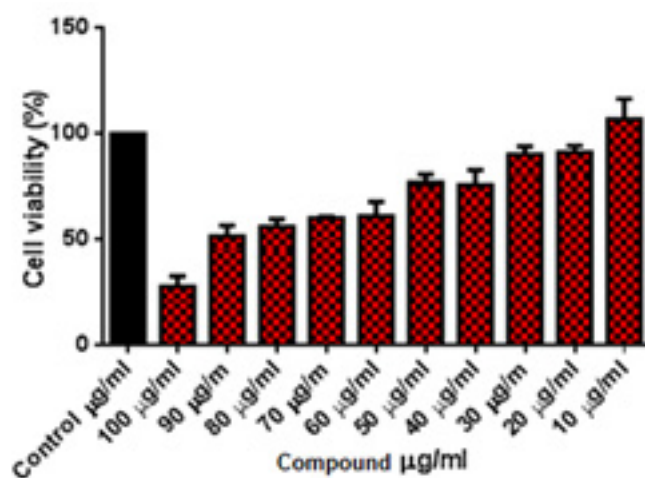
**Fig. 8.** Cell viability activity

HRBC membrane. The ethanolic extract of stem of *Andrographis echinoides* at different concentrations (20,40,60,80 and 100µg/ml) acts as good stabilization of HRBC as shown in table 6. *Andrographis* and diclofenac have more effectiveness and are illustrated in figure 6.

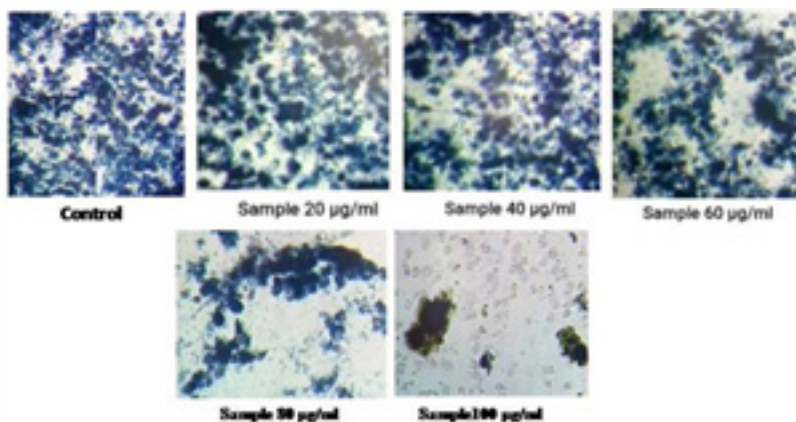
In a previous study, the HRBC method was analyzed under *in vitro* condition. Thus the medicinal plant extracts has the ability to stabilize the membrane for the slowness of inflammatory reactions by protecting the liberation of constituents of lysosomes of activated neutrophils as bactericidal

**Table 10.** Antifungal activity of ethanolic extract of *Andrographis echinoides*

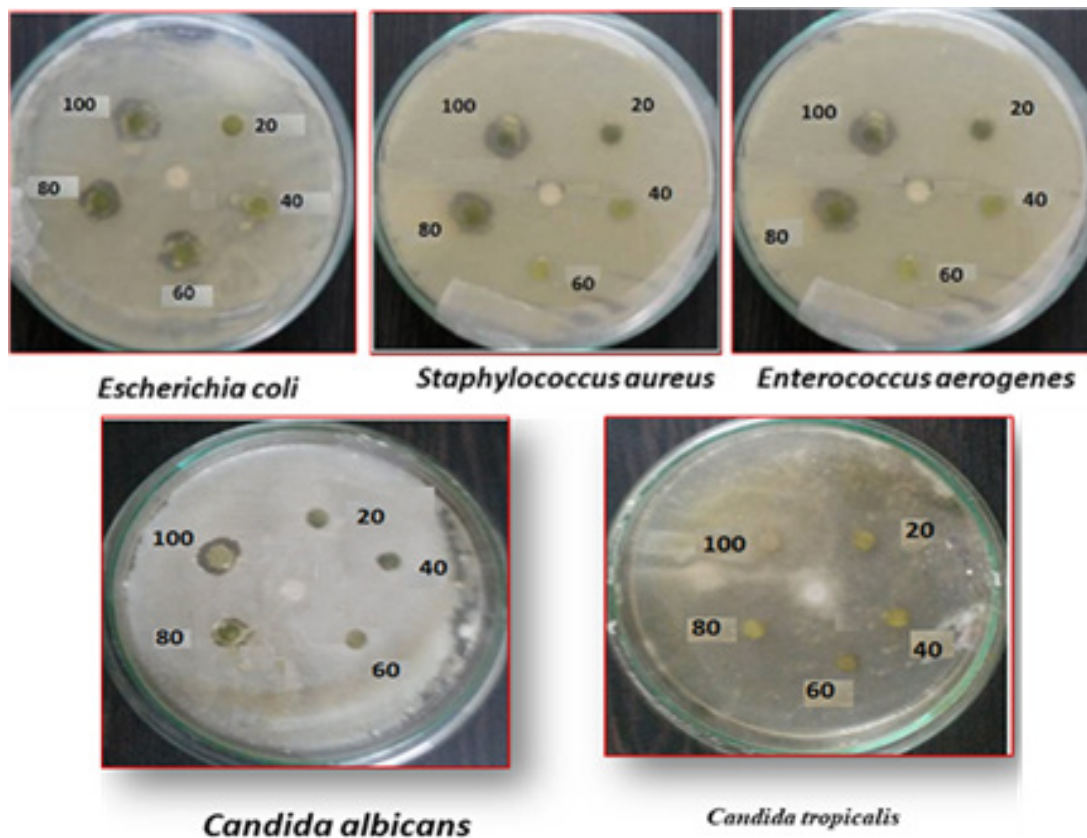
Plant extract	The concentration of ethanolic extract (µg/ml)	Organisms/zone of inhibition (mm)	
		<i>Candida albicans</i>	<i>Candida tropicalis</i>
Ethanol	20µg/ml	0	0
	40µg/ml	0	0
	60µg/ml	0	0
	80µg/ml	8	0
	100µg/ml	9	0
	10 µl/disc	0	0



**Fig. 9.** Cytotoxicity activity



**Fig. 10.** Formazan crystals in cells



**Fig. 11.** Antimicrobial activity

and protease enzymes, which leads to inflammation and damage of extracellular liberations<sup>20</sup>. The membrane stabilization mechanism of HRBC is majorly involved in the study of *Skimmia anquetilia*, *Gendarussa vulgaris*, *Thunnus alalunga*<sup>20, 29, and 30</sup>.

#### ***In vitro* Antioxidant activity of *Andrographis echioides* by DPPH assay method**

The ethanolic extracts of stem of *Andrographis echioides* contain the best antioxidant activity at high concentrations when compared with ascorbic acid. The plant showed 82.25% activity at 100 µl/ml at the same time ascorbic acid gave 85.03 at the same concentration (Table 7 and figure 7).

The ethanolic extract showed the highest DPPH scavenging activity showed the best antioxidant activity when compared to other extract<sup>31, 32</sup>. Antioxidant, cytoprotective and anti-inflammatory properties have been proved by *Andrographis echioides* with flavonoids, tannins, terpenoids, gums, cardiac glycosides and phosterols.

#### ***In vitro* cytotoxicity effects of samples**

The anti-cancer activity of *Andrographis echioides* was assessed by MTT assay. MCF-7 cell line was treated with different concentrations 20, 40, 60, 80, 100 µg/ml of plant samples and incubation for 24 hrs showed the percentage of cell viability (graph) of the cells in a dose dependent manner (figure 8) with IC<sub>50</sub> value of 58.46 µg/ml. This result is an agreement with a study carried out in previous study<sup>33</sup>. WHO reported the effectiveness of anticancer *D. innoxia* on human breast cancer cell line MCF-7. Thus the cytotoxicity study reveals the morphological change, characteristic, molecular and biochemical events present in an apoptotic cell. Another hand the cytotoxicity studies revealed that the *Andrographis echioides* exhibited dose-dependent toxicity on human breast cancer (MCF-7) cell lines. IC<sub>50</sub> value for MCF-7 cells showed a 50% noticed cell death is observed<sup>34</sup>.

The anticancer activity was performed by using this plant genus (*Andrographis paniculata*)

shown effective inhibition against the MCF-7 cell proliferation by using ethanolic extract<sup>35</sup>. Two different plants like *Andrographis paniculata* and *Silybummarianum* showed anticancer activity against five human cancer cell lines like human cervix (SiHa), human breast adenocarcinoma (MCF-7), ovary cancer cell line (ovcar-5), colon (HT-29) and liver (HepG2) by using a combination of this two plant<sup>36</sup>.

#### Antimicrobial activity of *Andrographis echioides*

Antimicrobial activity of *Andrographis* against microbes was performed by disc diffusion method as predicted in Table 9, 10 and plate 1-5. The extracts has well inhibitory activity of bacteria like *Escherichia coli* (11mm), *Enterococcus aerogenes* and *Staphylococcus aureus* (10mm) and fungi like *Candida albicans* (9mm), *Candida tropicalis* (0mm) at concentration 100µg/ml. At concentration 80mg/ml, the extracts exhibited antibacterial activity all the three bacteria, but was more susceptible against *Escherichia coli* (10mm), *Staphylococcus aureus* (9mm), *Enterococcus aerogenes* (9mm) and antifungal activity against *Candida albicans* (8mm). However, the crude extract observed as good inhibitory actions against pathogens with various concentrations (60, 80 and 100µg/ml). If concentration increases from 20-100µg/ml, then the inhibitory action also gets increased in this study.

The antibacterial activity against gram-negative and gram-positive strains by using ethanolic extract of *Andrographis echioides* showed maximum activity against gram-negative strains like *Salmonella typhi*, *Klebsiella pneumonia* and *Vibrio parahaemolyticus* when compared to control drug ampicillin and also showed least activity against gram-positive bacteria like *Bacillus subtilis* and *Staphylococcus aureus* and moderate inhibitory activity against *Aeromonas hydrophila*. *E.coli* when compared to control drug ampicillin<sup>37</sup>.

#### CONCLUSION

Thus the present work showed the ability of ethanolic extract of stem of *Andrographis echioides* shows the natural anti-arthritis, anti-inflammatory, anticancer activity under *in vitro* conditions and it may reduce the side effect of chemical drugs. More comprehensive studies related to this compound

will enhance pharmaceutical exploration in the field of cancer activities.

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#### Conflict of interest

The authors declare no conflict of interest.

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