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Evaluation of Phytochemical Content, Antibacterial Properties and Anti-inflammatory Activity of *Andrographis echioidies* (L) Nees

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Authors' contributions

This work was carried out in collaboration among all authors. Author DSR designed the study, performed the whole experiment, did the statistical analysis, wrote the protocol, wrote and edited the final draft of the manuscript. Authors SM and WM also did the whole experiment along with corresponding author. Author SD guides all the authors regarding this study. Author DCD identified the plant and did the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: There is much investigation with the *Andrographis paniculata* which is a very well-known medicinal plant, people were using this plant from ancient years, but this another species *Andrographis echioides* was not explored too much for many years. In this study we tried to find out the important phyto constitiuents, antibacterial activity and anti-inflammatory activity of this plant.
Aim: The main aim of the present study, was to screen phytochemical derivatives from an Indian medicinal plant *Andrographis echioides* (L) Nees and to evaluate the antibacterial potential, and anti-inflammatory activity also.

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Study Design: Various tests or experiment was done in this study according to the review of the literature with little modification, a preliminary phytochemical screening was carried out in the plant extracts using qualitative methods. The whole plant of *Anrographis echioides* was subjected to soxhlet extraction using organic solvent of methanol, acetone, and petroleum ether. The antibacterial activity of acetone, methanol and petroleum ether whole plant extracts of *Andrographis echioides* was carried out using agar well diffusion method, anti-inflammatory activity was checked using fresh egg albumin method, and a statistical analysis was done using SPSS to prove that there is a strong correlation between the activity of our plant sample and a standard commercial drug (NASID).
Place and Duration of the Study: The whole study was done in Panskura Banamali College, Vidyasagar University, Department of Biotechnology sponsored by BOOST, Louis Pasteur laboratory, West Bengal, India. The study was done between 17th August 2018 - 30th February 2019.
Results: All these tests were done triplicate and the mean value was taken. The phytochemical analysis reveals that the plant has some important phytoconstituents such as Tannins, flavonoids, phenolic compounds, glycosides and terpenoids. The Acetone and Methanol extracts are showed the best zone of inhibition against the severe pathogens such as *S. aureus* (A.E-33 mm, M.E-28 mm), *S. typhi* (A.E- 26.67 mm, M.E-28.33 mm). The methanolic extract of *Andrographis echioides* showed excellent anti-inflammatory activity comparing to the NASID (Non-steroidal anti-inflammatory drugs) Diclofenac sodium, it showed almost same activity.
Conclusion: Experimental findings reveal *Andrographis echioides* is the best herbal to control specially *S. typhi, E. coli, S. aureus, K. pneumoniae.* The phytochemical constituents which are responsible for many pharmacological activities, may be useful for the evolution of pharmaceutical and for the therapy of ailments, and also can used as a an potent anti-inflammatory natural remedy without any side effect.

Keywords: Andrographis echoides; phytochemical analysis; antibacterial activity; agar well diffusion; anti-inflammatory activity.

ABBREVIATIONS

NSAID : Non-steroidal Anti-inflammatory Drugs
CNH : Central National Herbarium
BSI : Botanical Survey of India
HPLC : High-pressure / Performance Liquid Chromatography
H_2So_4 : Sulphuric Acid
HCL : Hydrochloric Acid
MTCC : Microbial Type Culture Collection
MHA : Muller-Hinton Agar
µl : Micro liter
STD : Sexually Transmitted Disease
AE : Acetone Extract
ME : Methyl Extract
PE : Petroleum-ether Extract

1. INTRODUCTION

Since ancient times people were dependent upon plants for their foods, shelter and medicine. They used the plants for their daily life although chemical constituents of those plants unknown until that time. Before the age of Jesus Christ medicinal plants were listed and described in Ayurveda. After 500 AD. Many plants were listed as herbals in the renowned book Meteria Medica. Since ancient days people were habituated to use the plants for their health care knowingly or unknowingly. Basically, the rural people mainly used these plants for their traditional life. The tribal people have much more knowledge than others. Nowadays tribal medicines developed to a great extent [1]. India is the richest place for the source of medicinal plants and the people of India using these plants in three major systems: Ayurveda, Unani and Siddha. The knowledge of the chemical estimation and composition of the active principles of these herbals were meagre [2].

Although thousands of plants were used for herbal remedy, some of the plants are still remained un explored and in the black side of knowledge of science regarding their importance, activity, potentiality as well as phytoconstituents. We know the primary metabolites like carbohydrates, proteins and amino acids in the plants are very significant to maintain the life processes, but we have the very least knowledge about the secondary metabolites like alkaloids, terpenoids, flavonoids, tannins, saponins and steroids for their significance and disease healing property. These components may be present in

the root, stems, leaves, flowers, fruits, seeds, seed coat, fruit coat, and bark etc. separately or in combined form. We will concentrated to find out and isolate the potent bioactive compound present in this plant for further study to treat fatal diseases [3-6]. From a qualitative and quantitative point of view, the phytochemicals may vary from one part to others even in the same plant. Not only differ in their distribution but also lack of knowledge in their seasonal occurrence throughout the year [7]. Phytochemicals are at present key components for bioactive medicines, cultivated on a large scale and processed in a large scale in the pharmaceutical industry. Renowned scientists and researchers are engaged in the laboratory round the clock to focus their output [8-9]. Rheumatoid arthritis is a chronic, systemic inflammatory disorder that may cause inactive to many tissues and organs and the synovial joints [10,11]. Statistical analysis exhibits 1% population in the world affected by rheumatoid arthritis and female are affected three times than the male. Inflammation characterized by heat, redness, pain, swelling in joints. It may be the cause of the denaturation of proteins, noxious chemical or microbial infection [12]. Sometimes autoantigens tmay arise due to in vivo denaturation of proteins which may lead to inflammation [13]. Proteins are stabilized by different strong and weak bond, here the exact mechanism is that denaturation of protein was carried out [14]. New drugs or remedies have to be finding out to control the production of autoantigen and to inhibit the denaturation of protein for anti-arthritic or anti-inflammatory activity. Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used to control the inflammatory conditions which may have several adverse side effects especially gastric irritation leading to the formation of gastric ulcers [15-16].

There are much investigation with the *Andrographis paniculata* which is a very well-known medicinal plant, people were using this plant from ancient years, this plant is well known as kalmegh, Kalafath (English name “king of Bitters”). According to Indian ayurveda, *A.paniculata* “cools” and relives internal heat, inflammation and pain and it is used for dexotification [17-18], but this plant another species *Andrographis echioides* was not explored too much for few many years although this plant also have the same character as kalmegh, in this study we tried to find out the important phytoconstitiuents, antibacterial activity and anti-inflammatory activity of this plant. The plant *Andrographis echioides* (L) Nees, under the family *Acanthaceae* found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan, Assam, Bihar, West Bengal, and in various places in India, Uttar Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala [19]. It is a tribal traditional medicinal plant but remained beyond the knowledge of science until now. Detection of phytochemicals, as well as evaluation of antibacterial activity and anti-inflammatory activity, is the target of our investigation main from whole plant extracts in various solvents. The phytochemical analysis of *Andrographis echioides* (L) Nees has been done to certain extends following different chemical methods, the antibacterial activity has been done using Agar-well diffusion method and the anti-inflammatory activity was done using the egg albumin denaturation assay.

2. MATERIALS AND METHODS

2.1 Selection of Plant Material

Most of the tribal people have been using the *Andrographis echioides* (L) Nees for their common diseases regularly. On that context, we have chosen the material as our experimental tools. It was collected from beside the rail line field of panskura railway station, in the month of March, 2018, Purba medinipur (Longitude-87.749° ' east toLatitude-22º39'56'' north, and from the mean sea level 7 meters altitude), panskura, Purba Medinipur, West Bengal, India. In Fig. 1 the picture of the plant taken while the collection of plant material.

2.2 Description and Identification

Plant material has moderate stem height (30-100 cm), lance-shaped leaves, flowers are small pink, solitary arranged in racemes or panicles. Fruit is a capsule like around 2 cm containing many yellow-brown seeds flowering time is September to December. Plant material are having been identified from CNH (Central National Herbarium), BSI (Botanical Survey of India), Shibpur, Howrah; specimen no- ‘DSR-40’.

Taxonomic position [20-21]

Kingdom- Plantae
Sub-kingdom- Tracheobionta
Division- Magnoliophyta
Class- Magnoliopsida
Subclass- Asteridae
Order- Scrothulariales

Family- Acanthaceae
Genus- Andrographis Wall. Ex Nees
Species- *Andrographis echoids* (L.) Nees
Scientific name - *Andrographis echoides*

2.3 Surface Sterilization

A healthy, disease free fresh whole plants were selected for extraction. They are washed with distilled water, then 0.1% mercuric chloride was added to the whole plants for 20 seconds to done the surface sterilization. Again three times washed with distilled water.

2.4 Plant Material Extraction

After surface sterilization of whole plant part of *Andrographis echioides,* they were cut into small pieces, kept it few days for dried under shade. Then the air-dried plant material grounded into powder. The powdered material was extracted with various HPLC grade organic solvent like; acetone, ethanol, methanol, chloroform, petroleum ether hexane and distilled water using the soxhlet apparatus. About 10 grams of powder was loaded in the soxhlet extraction unit and exhaustively extracted using 100 ml of solvents such as acetone, ethanol, methanol, chloroform, petroleum ether, hexane and distilled water respectively at 60ºC for 12 hours. After that it was filtered with Whatman No-1 filter paper and use for various phytochemical analysis.

2.5 Phytochemical Screening

Phytochemical analysis of the test sample was carried out according to standard methods [22].

Test for Alkaloids: The alkaloids test was performed by the help of Wagner's reagent [100 ml of water contain 2 g of potassium iodide and 1.27 g of iodine]. The different solvent extracts of plant were added to this reagent and observed for the formation of the reddish brown precipitate.

Tests for Carbohydrates: 2 ml of various plant extracts were added with few drops of Molisch's reagent. Then the addition of concentrated H_2SO_4 (2 ml) to the test tube and allowed to wait for two-three minutes. After few minutes later a red or dull violet colour was formed of the two layers it indicate the positive test [23].

Test for Cardiac glycosides: In a test tube 5ml of each plant extract was taken and glacial acetic acid (2 ml) used to treat them and addition of few drops of ferric chloride solution. Then carefully sulphuric acid (1 ml) was added to the tested solution. At the junction of the two solutions a brown ring has appeared which indicates the presence of deoxy-ribose sugar characteristic of cardenolides. After that greenish ring may form to indicate the presence of Cardiac glycosides [23].

Test for Flavonoids: A portion of different crude extracts of the plant was added of 5 ml of dilute ammonium solution, followed by the addition of concentrated H_2SO_4. After that a yellow coloration may be formed in each of the plant extract indicates the presence of flavonoids.

Test for Phenols: 1 ml of various solvent extracts of plant kept into the different test tubes, 2 ml of distilled water and a few drops of 10% ferric chloride solution was added. Then blue or green colour was formed which indicates the presence of phenols [24].

Test for Phlobatannins: 2 ml of each plant extract was boiled with 1 ml of 1% aqueous hydrochloric acid to deposition of a red precipitate, which can indicate the presence of phlobatannins.

Fig. 1. Original picture of the *Andrographis echioides*, taken while plant sample collection

Test for Amino acids and Proteins: To perform the presence of amino acids and proteins, a few drops of ninhydrin solution treated with different plant solvent extracts and it placed with a boiling water bath for two 2 minutes and observed the formation of purple colour.

Test for Saponins: The formation of foam if 5ml of distilled water mixed with 2 ml of different plant extract in a test tube and it was shaken vigorously after that addition of few drops of olive oil indicates the presence of saponins.

Test for Tannins: In a test tube 1 ml of various plant extract was taken and then the addition of 1 ml of 0.008 (M) potassium ferric cyanide. After that, 1 ml of 0.02 (M) ferric chloride containing 0.1 (N) HCL was added and observed for blue – black coloration.

Test for Terpenoids: To perform the terpenoids test in a test tube different plant extract was kept and added concentrated H_2SO_4 and chloroform. After a few minutes later if a reddish brown precipitate formed then it shows positive test.

Test for Quinones: Formation of yellow precipitate if concentrated HCL added to the different plant extract indicates the presence of quinones.

2.6 Screening the Antibacterial Activity

2.6.1 Bacterial strains and culture conditions

The whole plant extracts of *Andrographis echioides* were screened against three pathogenic bacteria collected from the Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. The test organisms were *Escherichia coli* (MTCC-1687), *Staphylococcus aureus* (MTCC-3160), *Salmonella typhi* (MTCC-98). The nutrient agar slant culture was maintained at 37ºC and every 48 hours of transferring, and strains were stored in 50% glycerol stock at -20ºC.

2.6.2 Agar well diffusion method

The different solvent extracts of *Andrographis echioides* was used to determine the antibacterial activity by agar well diffusion method. Muller-Hinton agar (MHA) was used to perform this experiment. Test microorganisms were spread around in different MHA plates. 20µl of each extract was poured into each well and plates were incubated at 37ºC for 24 h. After 24 h zone of inhibition was measured in millimetre and the antibacterial results are taken [25]. The experiment was triplicated and the mean value was taken.

2.7 Screening the Anti-Inflammatory Activity *In vitro*

Methanol extract of *Andrographis echioides* was taken for the study of anti-inflammatory activity, this study was done by egg albumin denaturation assay [26]. In this assay fresh egg albumin was taken and inhibition of protein denaturation was observed compared to a commercial anti-inflammatory drug (NASID) i.e. Diclofenac sodium.

Control solution (50 ml): For making 50 ml of control solution, 28 ml of phosphate buffer was prepared at pH-6.4. Then 20 ml of distilled water and 2 ml of fresh egg albumin were added, volume made up to 50 ml.

Standard drug (50 ml): Here Diclofenac sodium used as commercial standard drug. To make these solutions different concentrations 100,200,400,800 and 1000 µg/ml concentration were made. Then at pH-6.4, 28 ml of phosphate buffer, 2 ml of egg albumin and 10 ml of various concentration of standard drug was taken [23].

Test solution (50 ml): For preparing the test solution of *Andrographis echioides* methanol extract, different concentration 100,200,400,800 and 1000 µg/ml were made. At pH-6.4, egg albumin along with phosphate buffer was mixed with methanol extract of *Andrographis echioides,* kept at room temperature for 20 minutes. At 660nm, in UV-vis spectrophotometer the absorbance was measured and the results were determined by the given formula: -

$$\text{Percentage of inhibition} = \frac{V_T}{V_C - 1} \times 100$$

Where, [V_T= Absorbance of test sample, V_C = Absorbance of control]

The entire experiment was triplicated and mean inhibition value was taken.

3. RESULTS AND DISCUSSION

The phytochemical, antibacterial and anti-inflammatory activity analysis of the whole plant of *Andrographis echioides* Represents interesting results where Quinone present in all the tested extracts whereas saponine, phlobatannins are absent, where the Acetone and methanol extracts shows excellent zone of inhibition against some dreaded pathogenic organism like *E. coli, S. typhi, and S. aureus* and the methanolic extract shows the good anti-inflammatory activity compared with the commercial anti-inflammatory drug i.e; Diclofenac sodium. The positive phytochemicals and potent antibacterial and anti-inflammatory agents of this plant lead to investigate further their pharmacological activities in the future against chronic Rheumatoid arthritis. Detail research on this plant can reveal a new era of phytochemistry, antimicrobial and anti-inflammatory study will provide more active

phytoconstituents, potent antibacterial agent and natural remedy against inflammation, for pharmacological significance. It should be finding new generations of drugs that may treat the various life threatening human diseases without having any side effects comparing to the available commercial drugs.

3.1 Preliminary Phytochemical Screening

The preliminary phytochemical analysis of *Andrographis echioides* in different extract showed the active compounds present in high concentration, such as flavonoids, phenolic compounds, glycosides and terpenoids where as saponins, phlobatannins, alkaloids, carbohydrates and amino acids and quinones are in low concentration (Table 1). Different tribal communities depend upon medicinal plants for their healthcare because it has great significance [27]. The plant extracts of the experimental tool phytochemicaliy analysed and different biochemical constituents have been proved their existence [28]. After careful analysis of the *Andrographis echioides* plant extracts revealed the presence of phytochemicals, such as proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, phlobatannins, terpenoids and alkaloids. During the present study, preliminary phytochemical analysis revealed a large amount of flavonoids, phenolic compounds, terpenoids and cardiac glycosides present in the different extracts of *Andrographis echioides* whole plant. The results are shown in Table 1.

3.2 Results of Antibacterial activity

The experiment of antibacterial activity using Agar Well diffusion method reveals an excellent zone of inhibition against some human pathogenic bacteria. It reveals that the best zone of inhibition was found against *S. aureus* using acetone extract and against *S. typhi* using methanol extract, the experiment was triplicated and the mean zone of inhibition was taken and for each zone of inhibition standard division was done, after that results were taken and tabulated in Table 2. Graphical representation was also done for the expression of various antibacterial activity through SPSS that was shown in Fig. 3. Fig. 2 is showing the zone of inhibition against some pathogenic microorganisms.

Fig. 2. Agar well diffusion method of different solvent whole plant extracts of *Andrographis echioides* against human enteric pathogens

Table 1. Preliminary phytochemical screening of *Andrographis echioides*

SL No.	Phytochemicals constituents	Acetone extract (A.E)	Methanol extract (M.E)	Petrolium ethere (P.E)
1	Alkaloids	-	-	+
2	Cardiac glycosides	-	-	-
3	Carbohydrates	+	+	+
4	Flavonoids	-	+	+
5	Phenols	-	-	-
6	Saponins	-	-	-
7	Sterol	-	-	+
8	Tannin	+	+	+
9	Terpenoids	-	-	-
10	Quinines	+	+	+
11	Protein	+	+	-

N.B [+ Positive, - Negative. A.E - Acetone extract, M.E- Methanol extract, P.E- Petroleum ether extract.]

Table 2. Antibacterial activity of different solvent whole plant extracts of *Andrographis echioides* against pathogenic organism

Whole Plant Extract Used	*Escherichia coli* (MTCC-1687)	*Staphylococcus aureus* (MTCC-3160)	*Salmonella Typhi* (MTCC-98)
Acetone (A.E)	27.66± 0.3 mm	33.00± 0.3 mm	26.66± 0.3 mm
Methanol (M.E)	26.00± 0.3 mm	28.00± 0.3 mm	28.33± 0.3 mm
Petroleum Ether (P.E)	12.66± 0.3 mm	-	14.00± 0.3 mm

[A.E = Acetone extract, M.E = Methanol extract, P.E = Petroleum ether extract, Values are mean of three replicates, ± values represent SD.]

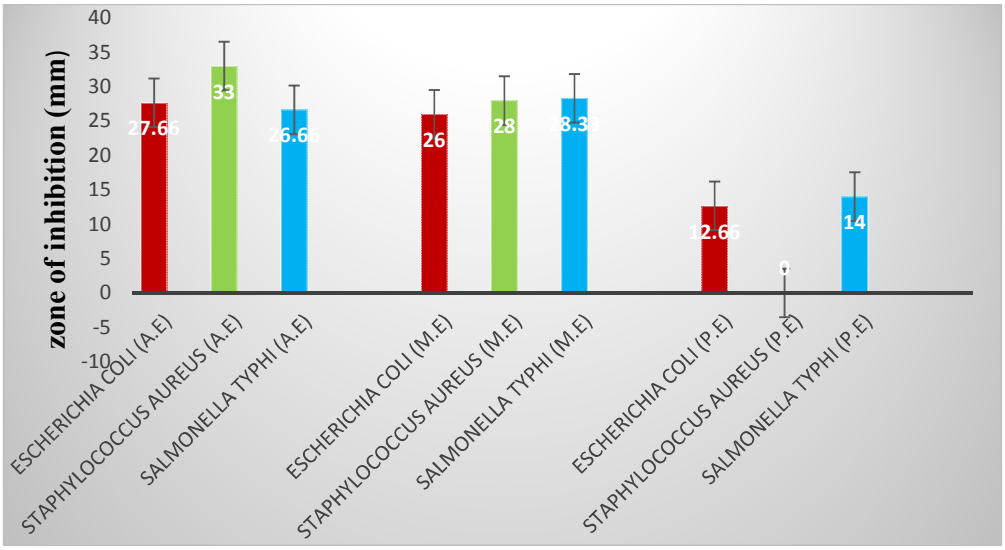

Fig. 3. Graphical representation of antibacterial activity of different solvent whole plant extracts of *Andrographis echioides* against human pathogenic microorganisms

[NB- A.E- Acetone extract, M.E- Methanol extract, P.E- Petroleum ether extract.]

Table 3. *In-vitro* anti-inflammatory activity of methanolic extract of *Andrographis echoides* on protein denaturation (Fresh egg albumin)

Concentration (µg/ml)	% inhibition of Diclofenac sodium	% inhibition methanol extract of *Andrographis echoides*
100	180	169
200	197.5	177
400	211.25	192
800	233.13	219
1000	234.37	225

3.3 Result of Anti-inflammatory Activity

During the *In vitro* anti-inflammatory activity study at different concentrations of *Andrographis echioides* methanolic extract and the standard drug diclofenac sodium showed very significant denaturation of egg albumin inhibition. Results and their comparison between sample and standard (Table 3 and Fig. 4). This experiment was triplicated and the mean value of inhibition was taken, a graphical representation was also prepared to compare the anti-inflammatory activity of *Andrographis echioides* methanolic extract and the activity of commercial standard drug diclofenac sodium.

3.4 Statistical Analysis

Statistical analysis was done using SPSS to find out that is there any correlation between diclofenac sodium inhibition (commercial drug) and methanolic extract of *Andrographis echoides*

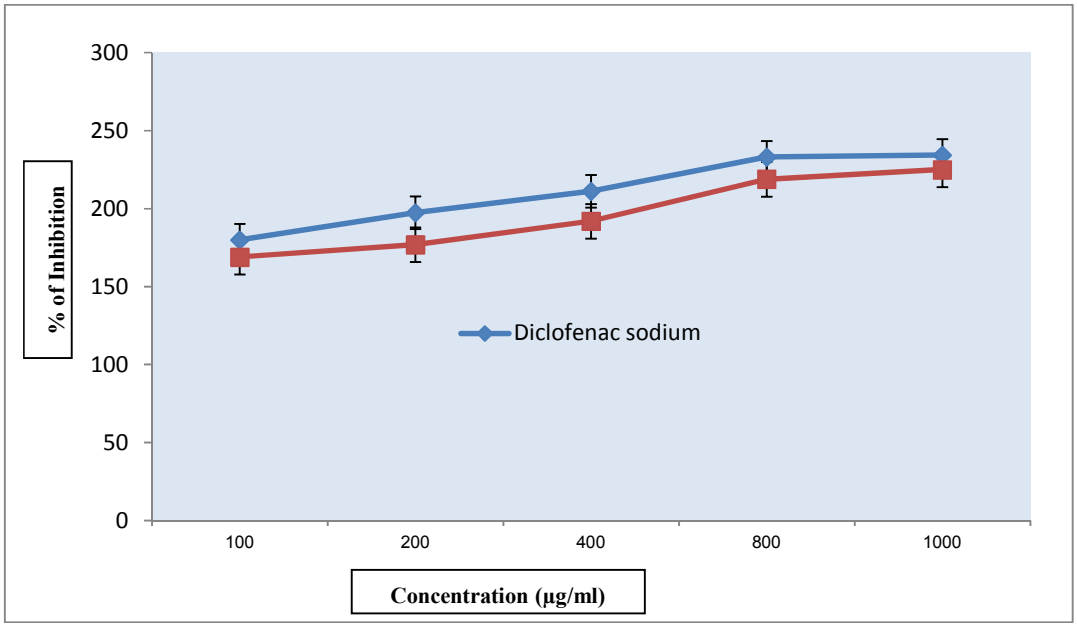

Fig. 4. Effect of methanolic extract *Andrographis echoides* on protein denaturation (Egg albumin)

Table 4. Results of Pearson correlation

Correlations			
		Inhibition of Diclofenac sodium	**Inhibition of Methanolic extract of Andrographis echoides**
Inhibition of Diclofenac sodium	Pearson Correlation	1	.981**
	Sig. (2-tailed)		.003
	N	5	5
Inhibition of Methanolic extract of Andrographis echoides	Pearson Correlation	.981**	1
	Sig. (2-tailed)	.003	
	N	5	5

***. Correlation is significant at the 0.01 level (2-tailed)*

N.B

Coefficient value	Strength of association
0.1 < | *r* | < .3	weak correlation
0.3 < | *r* | < .5	moderate correlation
| *r* | > .5	strong correlation

inhibition. Pearson correlation was done through SPSS to find out the correlation between them, results revealed that the 'r' value is 0.981 which means there are very strong correlation between Diclofenac sodium and the *Andrographis echoides* methanolic extract. The SPSS results are given (Table 4).

4. CONCLUSION

Experimental findings reveal *Andrographis echioides* is the best herbal to control especially *S. typhi, E. coli, S. aureus, K. pneumoniae* [24]. The phytochemical constituents which are responsible for many pharmacological activities, may be useful for the evolution of pharmaceutical and the therapy of ailments. This is the first ever experimental findings of antibacterial activity as well as anti-inflammatory activity, the results of these studies evaluate that this plant can be a natural remedy to combat chronic inflammation, viz., Rheumatoid Artheritis against some commercially available NASID (Non-steroidal anti-inflammatory drugs) which have too much

side effect. The correlation value also reveals that the methanolic extract of *Andrographis echioides* can act as a standard commercial drug (Diclofenac sodium). Demonstration of any biological properties in the globe deserves further investigation to develop new medicine that may help in combating several diseases in tropical countries to some extent. Further detail and accurate studies on this plant will explore a dynamic field of bioactive compound which is helping to develop new antibacterial medication as well as new anti-inflammatory drugs. It was also reported that this plant has antimicrobial activity against some sexually transmitted disease (STD) further work is going on to reveal the new drug against such STD pathogens.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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