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SCREENING OF *Andrographis Paniculata* EXTRACT FOR ANTIOXIDANT AND GENOTOXIC ACTIVITIES

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Abstract

Andrographis paniculata is an important medicinal plant which has been used to treat various ailments. The present study was undertaken to analyze the phytochemical compounds and evaluated the antioxidant and genotoxic potential of Andrographis paniculata leaves and whole plant extracts. Phytochemical compounds analyzed by qualitative and quantitative analysis of methanol extracts of the leaves and whole plant extracts which showed the presence of Alkaloids, Carbohydrate, Resins, Saponins, Flavonoid, Steroids, Glycosides and Tannin. Quantitative analysis were also conducted to determine the amount of Alkaloids, Flavonoids, Saponins and Tannin by HPTLC Finger printing methods in A. paniculata (A.P.) leaves and whole plant .The Antioxidant activity of A. paniculata Methanolic extract was evaluated by Fenton methods which showed the dose dependent Inhibition of TBARS formation. The Genotoxicity was evaluated by Micronucleus Assay. The dose dependent prevention of bone marrow micronucleus formation by Andrographis paniculata leaves and whole plant extracts was observed. Therefore, Andrographis paniculata leaves and whole plant extract caused antioxidant and genotoxic potential.

Keywords: Andrographis Paniculata; Phytochemical; Antioxidant; Micronucleus; HPTLC.

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1. Introduction

Plants have been an important source of medicine to living organism for thousands of years. Plants are contemplated not only as dietary supplement to living organism but also traditionally used for treatment of many health problems [1]. *Andrographis* is an important genus of the Acanthaceae family that is medicinally important taxa containing 26 species. *Andrographis paniculata* is also called as Kalmegh or "King of Bitters." This plant is an extremely bitter in taste that is used to treat liver disorders, bowel complaints of children, colic pain, common cold and upper respiratory tract infection [2,3,4]. It is also used for the treatment of snake bite, bug bite, diabetes, dysentery, fever, and malaria [5]. Whole plant leaves and roots are also used as a folklore remedy for different diseases in Asia and Europe. AP has been reported to have a broad range of pharmacological

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effects including anticancer, antihepatitis, antihyperglycemic, hepatoprotective, anti-inflammatory, antimicrobial, antidiarrheal, cardiovascular, cytotoxic, anti-HIV, immunostimulatory, and sexual dysfunctions[6]. Andrographis paniculata is used in Asia from centuries in traditional medicine to treat gastrointestinal (GI) tract and respiratory infectious diseases. It has been reported that Andrographis has a broad range of pharmacological effects[7]. It has been suggested to safe in controlled clinical trials report for treating upper respiratory tract infections. It also showed significant cardio protection by inducing antioxidant activity in myocardium[8]. Cytotoxic activity against cancer cell lines has been reported by Compounds of Andrographis paniculata [9]. Antimicrobial activity against eleven bacterial strains by ethanol extract of Andrographis paniculata have been reported [10]. Andrographolide have been reported to hypoglasmic activity in rats[11]. Antiulcer activity was reported in duodenal ulcer model in rats[12]. Hepatoprotective effect was reported on acetaminophen induced hepatotoxicity in albino rats[13]. An andrographolide was also reported to induce apoptosis in TD-47 human breast cancer cell line in a time and concentration-dependent manner by increase expression of p53, bax, immuno histochemical parameters such as caspase-3 and decrease expression of bcl-2[8]. Andrographis paniculata [14.] Dry leaf powder was reported to cause spermatogenesis, cessation of degenerative changes in the seminiferous tubules. The extract also produced significant muscarinic activity, which accounts for its antivenom effects[15].Many of the conditions commonly treated with Andrographis paniculata in traditional medical systems are important, which requires further investigations for benefit in cancer treatment.

2. Materials and Methods

Chemicals

All the Materials and Reagents used for the study were purchased from CDH, Renchem and Hi-Media Ltd., India.

Animals

The experimental study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight bearing, male *Swiss albino* mice. Animals were maintained under controlled conditions of temperature (24 ± 3^0C) and light (Light: dark, 10 hrs: 14 hrs.). The animals were provided with standard mice feed and tap water *ad libitum.*

Preparation of *Andrographis paniculata* Leaves and Whole Plant Extract

Plant material of *Andrographis paniculata* was collected and the specimen was authenticated by the botanist of Deendayal Research Institute, Chitrakoot, Satna, Madhya Pradesh(India). The non-infected leaves and whole Plant was washed, air dried, powdered and extracted separately using 50 % methanol in a separating funnel. Extract thus obtained were vacuum evaporated into powder. These extract was again dissolved in DDW immediately prior topical application.

3. Preliminary Phyto-Chemical Screening

Alkaloids

1 gram of dried powder was added with 10 ml of 1M-HCl and ultrasonicated for 15 min at 30ºC. The mixture was filtered and 3 ml of filtrate was treated with few drops of either Dragendorff's

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reagent or Mayer's reagent or Wagner's reagent. Orange red, creamy white or reddish brown precipitate indicated the presence of alkaloids.

Carbohydrate

Anthrone's test: Take 1ml of sample in test tube and take 1ml of distilled water in another tube as control. Add 2ml of anthrone reagent to all the tubes. Mix thoroughly all the content of the tube. Observe for color change in bluish green. That indicates all carbohydrate give test positive result.
Fehling's test: Filtrates were mixed with equal volume of Fehling's A and Fehling's B solutions and heated. Formation of brick red precipitate of cuprous oxide indicated the presence of reducing carbohydrate.

Proteins

Biuret test: To 0.5 mg of extract equal volume of 40% NaoH solution and two drops of one percent copper sulphate solution was added. The appearance of violet colour indicates that the presence of protein.

Resins

10 ml of distilled water were added to 1 g of dried powder, and ultrasonicated for 15 min at 30ºC. The mixture was filtered. Occurrence of turbidity showed the presence of rasins.

Saponins

About 0.5mg of the extract was shaken with five ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows that the presence of saponins.

Starch

Small quantity of extract was taken and add 2-3 drop of iodine solution on it. Observe the colour of solution. Blue black colour indicates the presence of starch.

Flavonoids

Ferric chloride test: Test solution when treated with few drops of Ferric chloride solution would result in the formation of blackish red color indicating the presence of flavonoids.

Alkaline reagent Test: Test solution when treated with sodium hydroxide solution, shows increase in the intensity of yellow color which would become colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

Steroids

2ml of chloroform and concentrated H_2SO_4 were added with the 5ml aqueous plant crude extract. In the lower chloroformlayer red color appeared that indicated the presence of steroids.

Glycoside

Borntrager s Test: About five mg of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl3 was added to the filtrate. Few drops of 10% NH3 were added to the mixture and heated. Formation of pink colour indicates that the presence Glycosides.

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Tannin

Lead acetate test: To the test solution, a few drops of 10% lead acetate solution were added. Precipitate formation indicated the presence of tannin.

Ferric chloride test: To the test solution, a few drops of ferric chloride solution were added.an intense green, purple, blue or black colour indicated the presence of Tannin.

4. HPTLC Fingerprint Profile

Sample preparation: Took 100g of drug in 250ml stoppered conical flask and extracted with 100ml alcohol for 24hrs. by maceration technique with occasional shaking. Decant the extract and make up to 100ml in volumetric flask.

Solvent system: *Toluene: Ethyl Acetate* (7:3)

Visualization: under 254nm; 366nm, and after derivatization 366nm and at visible Light (Image given in Annexure -1) & Major spots Rf Values given Annexure-2)

Derivatizing reagent: 5 *% Methanolic Sulphuric Acid*

5. Antioxidant Activity

Antioxidant activity of *Andrographis paniculata* Leaves and While Plant extract (10-100µg/ml) were determined by De-oxyribose Method (Fenton Reaction) of Halliwell et al., (1987). The hydroxyl radical attacked to deoxyribose and initiated a series of reaction that eventually resulted in the formation of Thiobarbeturic Acid Reaction Substances (TBARS).

6. Micronucleus Asaay

It was done by the method reported by Schmid (1975)[17], modified by Aron et al [18] and standardised by us [19] (Agrawal etal ,1998). In Micronucleus Assay, the extract of *Andrographis paniculata* Leaves and Whole plant at the volume of 0.2 ml at different dose level such as 1000. 1500, 2000 mg/kg body weight was injected 24 hrs before the treatment of Cyclophoshamide, to three animals. Singlre ip. Injection of 50 mg/kg Cyclophasphamide in 0.9% saline was injected 24 hours before the Andrographis extract treatment. The animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid (1975). After staining with May-Gruenwald and Giemsa Stain, a total 1000 cells were scored at the magnification of X 1000 (100 x 10x) for each group. The data are expressed as the average number of micro nucleated cells polychromatied erythrocytes cells (PCE) cells / animals.

7. Results and Discussions

The therapeutic properties of medicinal plants are perhaps due to the presence of various secondary metabolites that are phenols, flavonoids, alkaloids, glycosides, steroids, saponins etc. The Leaves and Whole plant of *Andrographis paniculata* extract have revealed the presence of Alkaloids, Carbohydrate, Resins, Saponins, Flavonoid, Steroids, Glycosides and Tannin. Protein and Starch are not present in the extract. The result Preliminary phyto-chemical screening of *Andrographis paniculata* Leaves & Whole plant Extrat shown in Table No.-1.

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Table 1: Preliminary phyto-chemical screening of *Andrographis paniculata* Leaves & Whole plant Extract

S. No.	Name of Experiments	Observation	Paniculata Leaves	Paniculata Whole Plant
1.	**Alkaloids**			
	Mayer' test	Yellow colour appear	Present	Present
	Wagner's test	Brown colour appear	Present	Present
	Dragendorff's test	Orange colour appear	Present	Present
2.	**Carbohydrate**			
	Anthrone's test	Dark green colour appear	Present	Present
	Fehling's test	Brick red colour appear	Present	Present
3.	**Proteins**			
	Bieuret's test	Green colour appear	Absent	Absent
	Millon's test	White ppt are not appear	Absent	Absent
5.	**Resins**	Turbidity are seen	Present	Present
6.	**Saponins**	Honey comb – like structure are form	Present	Present
7.	**Starch**	Red colour is formed	Absent	Absent
8.	**Flavonoid**		Present	Present
	Ferric chloride test	Reddis pink colour is appear	Present	Present
	Alkaline reagent test	On addition of dilute acid yellow colour disappear	Present	Present
9.	**Steroid**			
	Salkowski's reaction	A red colour is disappear in the chloroform layer	Present	Present
10.	**Glycoside**			
	Borntrager's Test	Colour is change	Present	Present
11.	**Tannin**	Greenish colour appear	Present	Present
	a) Lead acetate Test	Reddish brown bulky ppt. are formed	Present	Present

Quantitative Phyto-chemical Analysis

The quantitative analysis that shows the percentage of Alkaloids, Saponins, Flavonoids, and Tannin present in extract. The result of Quantitative Phyto-chemical Analysis of *Andrographis paniculata* Whole plant & Leaves extract shown in Table No.-2.

Table 2: Quantitative Phyto-chemical Analysis of *Andrographis paniculata* Whole plant & Leaves extract

S. No.	Name of tests	*Andrographis paniculata* Leaves	*Andrographis paniculata* Whole Plant
1	**Alkaloids**	1.7642%	2.28%
2	**Flavonoids**	12.13%	13.02%
3	**Saponins**	3. 51%	3. 79%
4	**Tannin**	5.12%	5.39%

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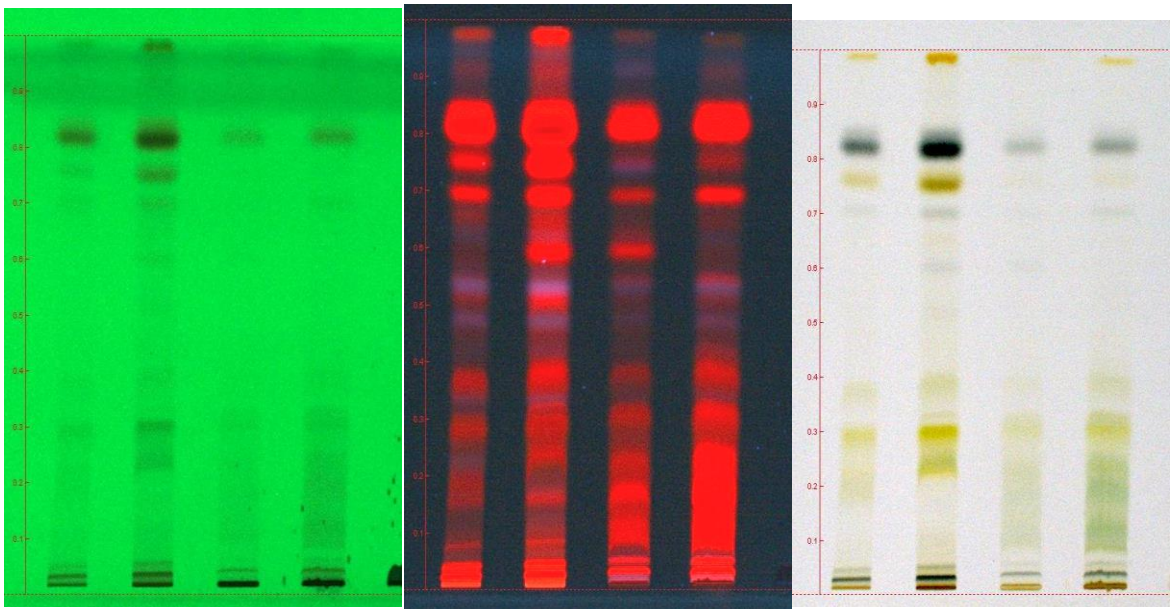
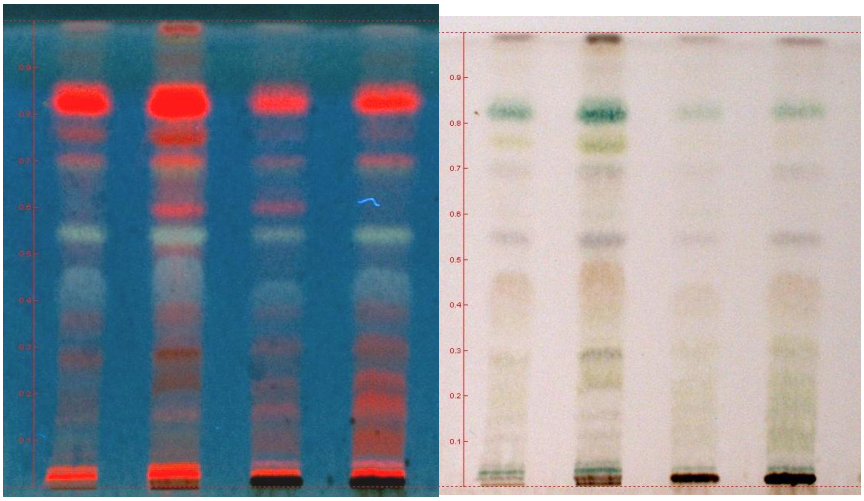
HPTLC Fingerprint

In HPTLC (High Performance Thin Layer Chromatography) fingerprinting analysis bands were observed on the HPTLC plates and the Rf values were calculated, where Rf value is Retention factor value. The observed fingerprint, rather than the presence of the compounds, represents a unique pattern and is given by a set of Rf values and the specific colours observed for these compounds. (Fig 1 & 2)



A B C D A B C D A B C D At
254 nm before derivatization At 366 nm before derivatization At Daylight before derivatization



A B C D A B C D
At 366 nm after derivatization At Day light after derivatization

Track A: Methanol extract; Track B: Ethanol extract; Track C: Benzene extract and Track D: Chloroform extract

Figure 1: HPTLC Fingerprint Profile of Andrographis paniculata (Leaves)

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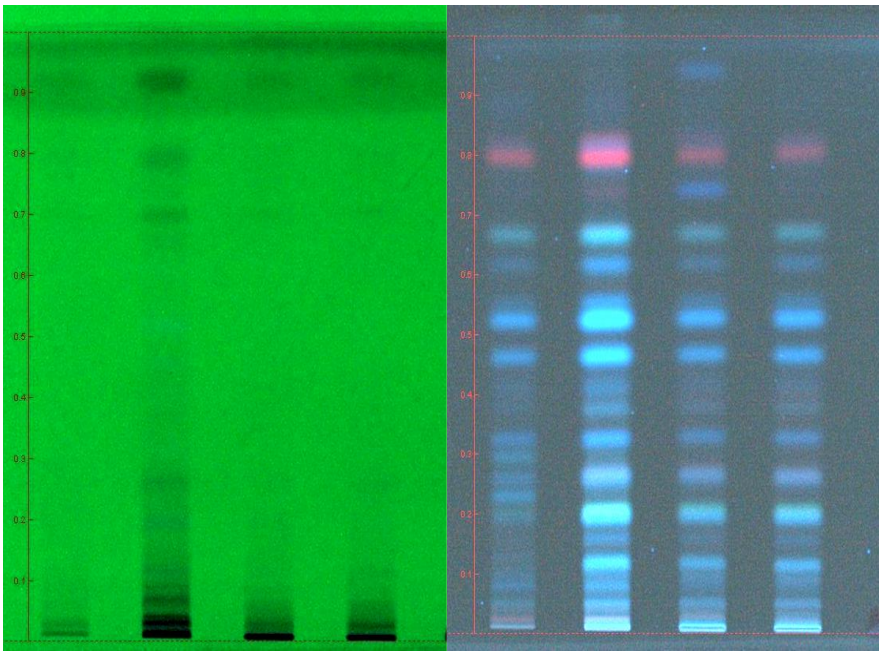
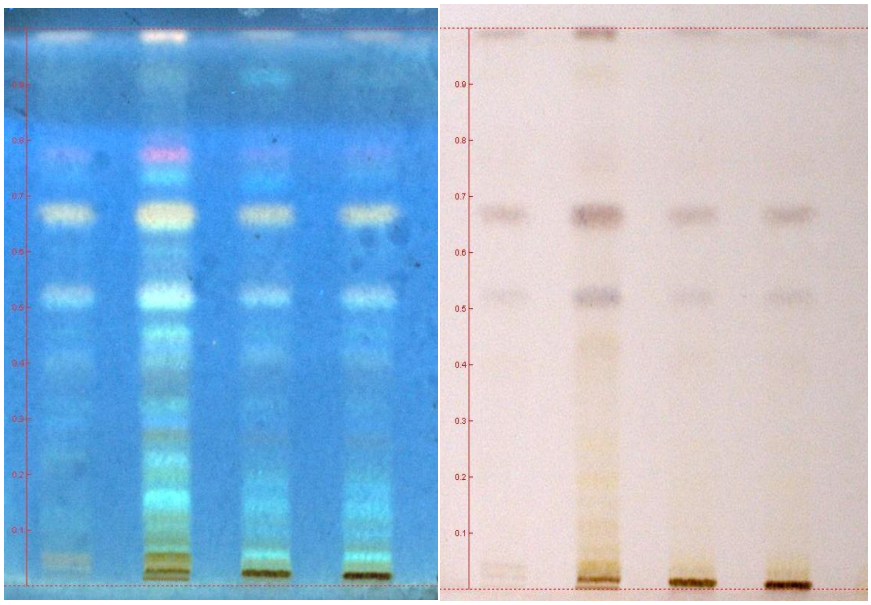


A B C D **A B C D**

At 254 nm before derivatization **At 366 nm before derivatization**



A B C D **A B C D**

At 366 nm after derivatization **At Day light after derivatization** **Track A: Methanol extract; Track B: Ethanol extract; Track C: Benzene extract and Track D: Chloroform Extract**

Figure 2: HPTLC Fingerprint Profile of Andrographis paniculata (Whole plant)

8. Antioxidant Activity

The free radical scavenging capacity of the methanolic extract of *Andrographis paniculata* Leaves and Whole palnt extract were determined by using TBARS method. *A. paniculata* showed

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antioxidant activity when compared with Ascorbic acid that is used as positive control. The dose of different concentration of *A. paniculata* Leaves and Whole plant extract were observed. The TBARS values were compared with Ascorbic acid. (Table No.-3)

Table 3: Antioxidant Activity of Methanolic extract of *Andrographis paniculata* and Ascorbic acid as standard

S.No.	Concentration (µg/ml)	% of Inhibition (TBARS)		
		Ascorbic acid	*A.paniculata* Leaves extract	*A.paniculata* Whole plant extract
1	10	18.54	10.11	4.25
2	20	23.09	21.14	10.36
3	30	30.71	30.96	19.17
4	40	34.67	34.38	31.2
5	50	42.58	33.25	21.99
6	60	50.71	37.36	37.96
7	70	56.71	47.98	46.25
8	80	61.13	41.54	53.58
9	90	71.77	52.05	59.33
10	100	79.47	65.04	81.06

9. Micronucleus Assay

In the Micronucleus Assay Cyclophasphamide used as clastogenand anticlastogenic effect of *A. paniculata* has been observed in mice bone marrow cells (Table 3). A reduce number of micronuclei were seen in *A. paniculata* Leaves and Whole plant extract along with Cyclophosphamide as compared the Cyclophosphamide alone. The dose of 1000, 1500, 2000 mg/kg body weight showed the reduction of micronucleus formation in PCE cell of bone marrow. The PCE/NCE ratio of *A. paniculata* Leaves and Whole palnt was increased as compare to Cyclophasphamide alone. (Table No. - 4)

Table 4: Effect of *A. paniculata* leaves and whole plant extract on Micronucleus (MN) formation induced by Cyclophosphamide (CP) in bone marrow cells of *Swiss albino* mice.

Groups	Treatment Doses (mg/kg body weight)	MNPCE ± SEM	PCE/NCE Ratio ±SEM	Protection % of CP induced MN formation
I	**Cyclophosphamide Alone (50mg/kg b.wt)**	4.25±1.5	0.76±0.02	-
***A. paniculata* leaves**				
II	***A. paniculata* leaves alone (1000mg/kg b.wt)**	0.5±0.57	0.77±0.02	-
III	***A. paniculata* leaves (1000mg/kg b.wt) + CP (50mg/kg b.wt)**	2.0±0.81	0.88±0.08	52.95
IV	***A. paniculata* leaves (1500mg/kg b.wt) + CP (50mg/kg b.wt)**	1.0±0.57	1.02±0.01	76.48

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V	***A. paniculata* leaves (2000mg/kg b.wt) + CP (50mg/kg b.wt)**	0.5±0.57	1.10±0.04	88.24
***A. paniculata* whole Plant**				
VI	***A. paniculata* whole Plant Alone (1000mg/kg b.wt)**	0.5±0.57	0.67±0.99	-
VII	***A. paniculata* whole Plant (1000mg/kg b.wt) + CP (50mg/kg b.wt)**	2.25±0.5	0.76±0.11	47.06
VIII	***A. paniculata* whole Plant (1500mg/kg b.wt) + CP (50mg/kg b.wt)**	1.5±0.57	1.21±0.41	64.71
IX	***A. paniculata* whole Plant (2000mg/kg b.wt) + CP (50mg/kg b.wt)**	1.0±0.81	1.06±0.05	76.48

PCE – Polychromatic erythrocytes, NCE – Normochromatic erythrocytes, MNPCE – Micronucleated Polychromatic erythrocytes

10. Discussion and Conclusions

The preliminary phytochemical screening tests may be helpful in the identification of the bioactive principles and may lead to drug discovery and development. These tests facilitates their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The secondary metabolites or phytochemicals such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols etc have medicinal value for eg. Saponins have hypotensive and cardiode pressant properties. Glycosides used for the treatment of congestive heart failure and cardiac arrhythmia[20] Phenolics are major group of compounds that are flavonoids and Tannin, that act as free radical scavengers or primary antioxidants. Our phytochemical screening of Andrographis paniculata. give ideas regarding various secondary metabolites present in leaves and whole plant. Phytochemical showed the presence of glycosides, steroidal compounds, flavonoids, and saponins. Qualitative densitometric HPTLC fingerprint profile of methanolic extract can provide standard fingerprints and can be used as a reference for the identification and quality control of the fruit. The present study will provide the information with respect to identification and authentication of Andrographis paniculate.

In present study leaves and whole plant of *Andrographis paniculata* methanolic extracts showed antioxidant activity in dose dependent manner. The antioxidant activity of methanolic extract of leaves of *A. paniculata* was reported by decreased tissue malondialdehyde level and increased SOD levels due to its antioxidant and cerebro protective activity against cerebral infarction in Type II diabetic animal model [21]. The concentration ranged from 10 to 100µg/ml. The reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity. The presence of reductones, break the free radical chain by donating a hydrogen atom. Reductones (i.e. antioxidants) presence in the sample extracts might cause the reduction of Fe3+/ Ferric Cyanide complex to Ferrous form which can be monitored by Spectrophotometer[22]

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The present study showed that Andrographis paniculata extract caused the dose dependent inhibition of micronucleus formation in bone marrow cells of mice. This plant can be studied furthermore to know their biological effects which could be a helpful in the treatment and controlling of various diseases.

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