Int J Pharm Bio Sci 2015 April; 6(2): (P) 475 - 484

Research Article **Medicinal chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY, ANTIOXIDANT ACTIVITY, ANTICOAGULANT ACTIVITY AND FIBRINOLYTIC ACTIVITY OF *LEAVES OF ANDROGRAPHIS PANICULATA(LEAF)*

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ABSTRACT

Medicinal plant based drugs have an added advantage of being simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action. They contain active constituents that are efficient in the treatment of many human diseases. The present study was an attempt to assess few antibacterial, antioxidant, anticoagulant and fibrinolytic activity of *Andrographis paniculata.* The phytochemical screening of *A. Paniculata* (leaf) revealed the presence of flavanoids, glycosides, saponins, phenols, tannins and steroids. . The plant part (leaf) exhibited antibacterial and antioxidant activities that varied from solvent to solvent. The study also revealed that ethanolic extract exhibited significant antimicrobial activity, whereas the methanolic extract exhibited antioxidant activities compared to other organic solvents and aqueous extracts. The aqueous extract exhibited significant anticoagulant activity compared to ethanol and methanol extract.In fibrinolytic activity, the ethanol and methanol extract exhibited significance result whereas in aqueous extract there was no fibrinolytic activity.

KEYWORDS: **Antimicrobial activity, Antioxidant activity, anticoagulant activity, fibrinolytic activity and phytochemical constituents.**

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INTRODUCTION

In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities' uses about 800 plant species for curing different diseases[1]. Currently 80% of the world population depends on plant-derived medicine for human alleviation because of its fewer side effects. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources[2]. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[3].Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.[4] An anti-microbial is a substance that kills or inhibits the growth of microorganism such as bacteria, fungi and protozoan. The role of antioxidants is to neutralize the excess of free radicals and to protect the cell against their toxic effects and to contribute to disease prevention. Anticoagulants (antithrombics, fibrinolytic, and thrombolytics) are a class of drugs that work to prevent the coagulation (clotting) of blood. Such substances occur naturally in leeches and blood-sucking insects. A group of pharmaceuticals called anticoagulants can be used i*nvivo* as a medication for thrombotic disorders. Some anticoagulants are used in medical equipment such as test tubes, blood transfusion bags, and renal dialysis equipment. Fibrin is an insoluble protein involved in blood clotting; the primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause. These anticoagulants are used to treat patients with Deep-Vein Thrombosis (DVT), Pulmonary Embolism (PE) and to prevent emboli in patients with atrial fibrillation (AF) and mechanical prosthetic heart valves[5]. Therefore, the characterization of extract of medicinal plants is necessary due to its numerous benefits to science and society. Hence, the present investigation was taken up with an objective to evaluate the phytochemical screening, antimicrobial, antioxidant, anticoagulant and fibrinolytic activity of *Andrographis paniculata.*

MATERIALS AND METHODS

Sample Collection

The medicinal plants were collected locally from the farm lands of sulur area, Coimbatore district (India). The plants collected were identified botanically in Manian Laboratory,Coimbatore. Fresh and tender leaves of selected plant were used for phytochemical analysis. Fresh leaves of medicinal plant namely *Andrographis paniculata* were collected in sterile bags and carried to the laboratory.

Preparation of plant material

The fresh leaves were washed with tap water and then thoroughly cleaned with distilled water and shade dried for a week. Then the dried leaves were grinded to a fine powder by using mortar and pestle. 10 gms of the powder was taken and macerated in 100 ml of different solvents methanol, ethanol and aqueous. They were kept at room temperature for 24 hours .Thereafter the mixtures were filtered by using Whatmann filter paper no .1.

Screening of preliminary phytochemical analyses

Phytochemical examinations were carried out for all extracts as per the standard methods [5].

1. Detection of Alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) ***Mayer's Test***

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) Wagner's Test

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide) Formation of brown/reddish precipitate indicates the presence of alkaloids.

2. *Detection of Flavonoids*
a*) Alkaline Reagent Test*
Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

c) Lead acetate Test
Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids

2. Detection of Glycosides
Extracts were hydrolyzed with diluted Hcl, and then subjected to test for glycosides.

a) Modified Borntrager's Test
Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer were separated and treated with ammonia solution. Formation of rose-pink color in the ammonia layer indicates the presence of anthranol glycosides.

b) Legal's Test
Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

4. *Detection of Saponins*
a) Froth Test
Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1cm layer of foam indicates the presence of saponins.

b) Foam Test
0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. *Detection of Phenols*
Ferric Chloride Test
Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

6. Detection of Tannins
Gelatin Test
To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

7. Test for Sterols
a) *Libermann puchards Test*
5ml of unsaponifiable fraction were dissolved in 2ml chloroform and 2ml of acetic anhydrate. Then add 2drops of concentrated sulphuric acid to the above solution.

B) *Salkowskis Test*
2ml of extract was dissolved in 3ml of chloroform and 2 drop of concentration sulphuric acid was added along the test tubes.

8*. Detection of Aminoacids*
Ninhydrin Test
To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color in
dicates the presence of amino acid.

9. Detection of Diterpenes
a) Copper acetate Test
Extracts were dissolved in water and treated with 3- 4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes.

10. Detection of Anthraquinones
Borntragers Test: 2ml of sample were heated with 2ml of $FeCl_3$ and 2ml of concentration hydrochloric acid was added. The solution was cooled and filtered and the filtrate was shaken with 2ml of diethyl ether. Further this was extracted with 1ml of strong ammonia and the colour was noted.

Antimicrobial activity of Andrographis paniculata
Test microorganism used
1. *Staphylococcus aureus* MTCC3381
2. *Escherichia coli* MTCC739

Preparation of inoculum
The test organisms were sub cultured by streaking them on nutrient agar, followed by incubation for 24 hr at 37 °C. Several colonies of each bacterial species were transferred to sterile nutrient broth. The suspensions were

mixed for 15 sec and incubated for 24 hr at 37 °C on an orbital incubator shaker. Working concentration of the microbial suspension was prepared in 3 mL of sterile saline to turbidity equivalent to 0.5 McFarland scale (i.e., adjusting the optical density to 0.1 at 600 nm) yielding a cell density of 1-2 × 105 CFU/ mL Nutrient Agar (NA) plates were seeded with 8 hr broth culture of different bacteria. In each of these plates, wells were cut out using sterile cork borer. Using sterilized dropping pipettes, different concentrations (250, 500, 750 and 1000 µg/well) of sample was carefully added into the wells and allowed to diffuse at room temperature for 2 hr. The plates were then incubated at 37 °C for 18–24 hr. Gentamicin (10µg) was used as positive controls and 50% ethanol/methanol as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone [6,7].

ANTIOXIDANT ACTIVITY

FREE RADICAL SCAVENGING ACTIVITY ON DPPH

The antioxidant activity of the sample was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH, according to the method of Blois (1958). The sample extracts at various concentrations (200 - 1000µg) was taken and the volume was adjusted to 100 µl with methanol. 5 ml of 0.1 mM methanolic solution of DPPH were added and allowed to stand for 20 min at 27 °C. The absorbance of the sample was measured at 517 nm using UV – Visible spectrophotometer [8,9]. Percentage radical scavenging activity of the sample was calculated as follows:

% DPPH radical scavenging activity = (control OD-sample OD / control OD) × 100

The analysis was performed in triplicate. The sample concentration providing 50% inhibition (IC50) under the assay condition were calculated from the graph of inhibition percentage against sample concentration[10,11].

ANTICOAGULANT ACTIVITY

Anticoagulant activity of the plant (leaf) extract studied by using the method of Naraspur (1997).A clear venepuncture was done; 3ml of blood were drawn in a syringe and transferred in to 4 test tubes containing 0.5ml of blood. A clean tile was taken; the ethanolic, methanolic extract and aqueous extract of *Andrographis paniculata* were taken in test tubes. Place a clotted blood in the tile. Add ethanolic extract of the plant and observed for anticoagulation and place a 0.5ml of blood in the tile and add methanolic extract of the plant (leaf) and observed for the anticoagulation. Place a 0.5ml of blood in the tile and add aqueous extract of the plant and observed for the anticoagulation[12].

FIBRINOLYTIC ACTIVITY

Fibrinolytic activity of the plant (leaf) extract studied by using the method of Naraspur (1997).A clear venepuncture was done, 3ml of blood were drawn in a syringe and transferred in to 4 test tubes containing 0.5ml of blood. A clean tile was taken, the ethanolic, methanolic extract and aqueous extract of *Andrographis paniculata* was taken in a test tubes.Place a clotted blood in the tile. Add ethanolic extract of the plant (leaf) and observed for fibrinolysis and place a 0.5ml of blood in the tile,add methanolic extract of the plant and observed for the fibrinolysis and place a 0.5ml of blood in the tile and add aqueous extract of the plant and observed for the fibrinolysis[12].

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

The results of the preliminary phytochemical analyses were carried out in extracts of *Andrographis paniculata* medicinal plant. The experiment showed the presence of secondary metabolites such as alkaloid, glycosides, flavonoids, saponins, tannins, steroids, protein, amino acid and phenol.

Table 1
Preliminary phytochemical analysis of Andrographis paniculata

S.No.	Secondary Metabolites	Ethanol	Methanol	Chlorofom	Aqueous
1.	Alkaloids	_	_	+	_
2.	Flavonoids	_	+	+	+
3.	Glycosides	_	+	+	_
4.	Saponins	_	+	+	+
5.	Phenols	+	+	+	+
6.	Tannins	+	+	_	+
7.	Steroids	+	+	+	_
8.	Amino acid	_	_	+	+
9.	Diterpenes	_	+	_	_
10.	Anthraquinones	_	_	_	_

(+indicates presence, - indicates absence)

The results of the phytochemical analyses of *Andrographis paniculata* are shown in Table 1. The phenols in ethanol, methanol, chloroform and aqueous are found to present in all solvents. The Anthroquinone compound shows absence in all the solvents. Hence the phytochemical screening reveals that Aqueous, Methanol and Chloroform extract shows high secondary metabolites. Thus the preliminary screening analysis is helpful in the detection of bioactive compounds and lead to the discovery and development of novel drugs[13,14] .

ANTIMICROBIAL ACTIVITY

Antimicrobial activity is used to test whether the leaf extract has capability to control the growth of the microorganism. The present approach is the microbiological study involving antibiotic sensitivity test employed to the two microorganisms. The zone of inhibition has been obtained for all the microorganism are shown in the figure, the microorganism used are

1. Staphylococcus aureus MTCC3381
2. *Escherichia coli* MTCC739

Antimicrobial activity of ethanol and methanol extract of *Andrographis paniculata* against *Staphylococcus aureus* and *Escherichia coli* was tested.

Table 2
Antimicrobial activity of Andrographis paniculata

Sample	Conc. (µg/ml)	Zone of inhibition (mm)	
		Staphylococcus aureus	***Escherichia coli***
	250	0.00 ± 0.00	0.00 ± 0.00
50 % Ethanol	**500**	11.00 ± 0.00	10.00 ± 0.00
	750	13.33 ± 0.58	10.67 ± 0.58
	1000	14.33 ± 0.58	13.33 ± 0.58
	250	10.00 ± 0.00	0.00 ± 0.00
50 % Methanol	**500**	10.67 ± 0.58	10.00 ± 0.00
	750	11.33 ± 0.58	11.33 ± 0.58
	1000	11.67 ± 0.58	12.67 ± 0.58
Gentamicin	**10**	17.33 ± 0.82	15.17 ± 0.75

Values are means of three independent analysis ± Standard Deviation (n=3)

The result obtained, the zone of inhibition was recorded at four concentrations of 250, 500, 750, 1000 µg/ml in Table 2. From the figure 3-6 it was clearly observed, the well diffusion assay on the ethanol showed an inhibit increasing inhibitory effect than methanol on bacterial growth with increasing concentration of an extraction.

Figure 3
50% Ethanolic extract versus Escherichia coli

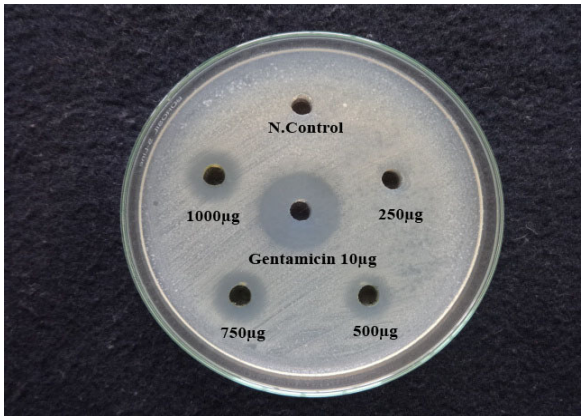


Figure 4
50% Ethanolic extract versus Staphylococcus aureus

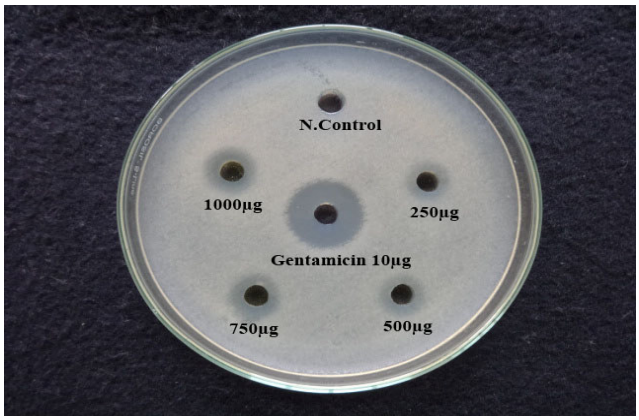


Figure5
50% Methanol extract versus Escherichia coli

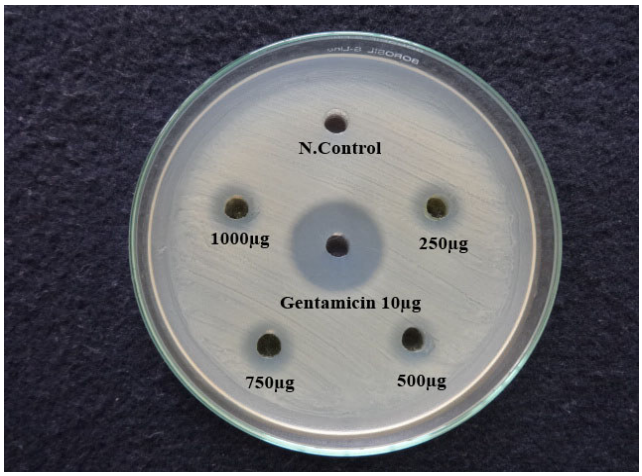
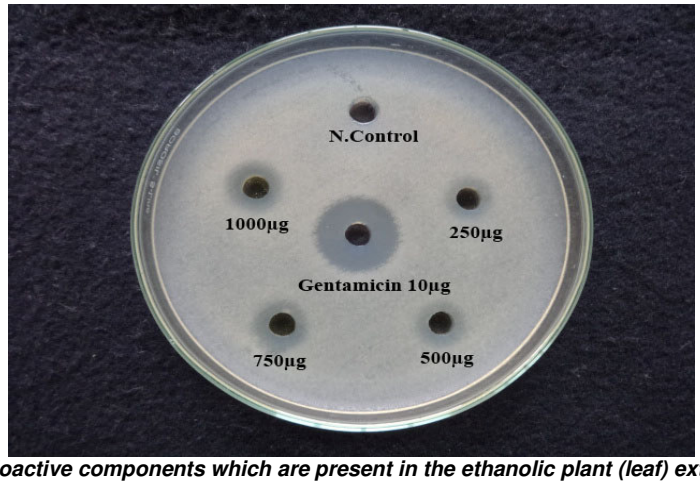


Figure 6
50%Methanol extract versus Staphylococcus aureus



This may be due to the potent bioactive components which are present in the ethanolic plant (leaf) extract of Andrographis paniculata.

ANTIOXIDANT ACTIVITY

Free radical scavenging assay

Increased absorbance of the reaction mixture indicates increased free radical activity ascorbic acid is taken as a standard

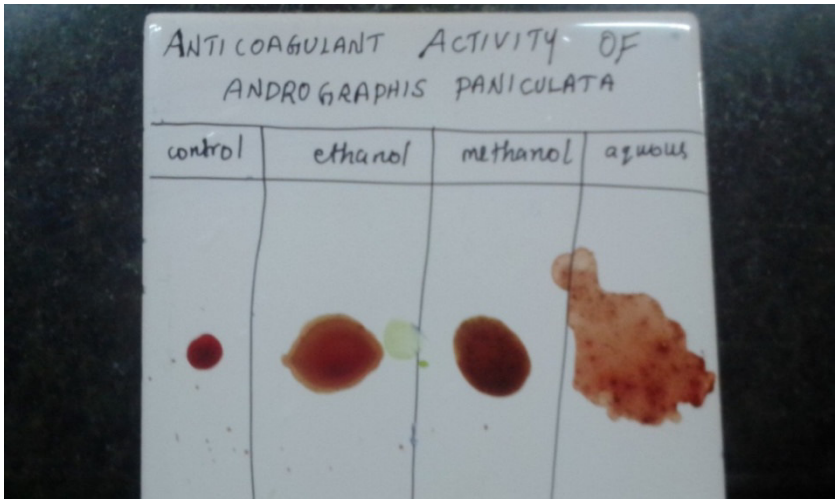
Table 3
DPPH radical scavenging activity

Sample	Concentration (µg)	Percentage activity (%)	IC50 (µg/ml)
50 % Ethanol	200	27.78± 0.14	
	400	49.66± 1.60	159.75± 0.88
	600	68.22± 2.65	
	800	84.96± 0.24	
	1000	95.97± 0.31	
50% Methanol	200	29.16± 3.60	
	400	54.70± 2.02	
	600	78.54±1.30	148.82± 1.33
	800	94.51 ± 0.19	
	1000	96.96 ± 0.09	
Ascorbic acid	2	7.75 ± 0.61	
	4	18.98 ± 0.38	
	6	31.98 ± 2.42	3.61 ± 0.20
	8	43.18 ± 2.67	
	10	54.62 ± 2.60	

The ethanolic and methanolic extracts were taken for assessing antioxidant activity. The results were presented in Table 3.The antioxidant activity mainly non enzymatic (Ascorbic acid) was estimated by colorimetric assay,the radical scavenging activity and total antioxidant activity was recorded maximum for ethanolic extract when compared to other organic solvents and aqueous extracts[15].Further, the significant antioxidant activities recorded and high amount of total phenolic content present in the ethanolic extract of of *Andrographis paniculata* are responsible for preventing the damage caused by the free radicals by scavenging them and protect the living organisms.

ANTICOAGULANTACTIVITY

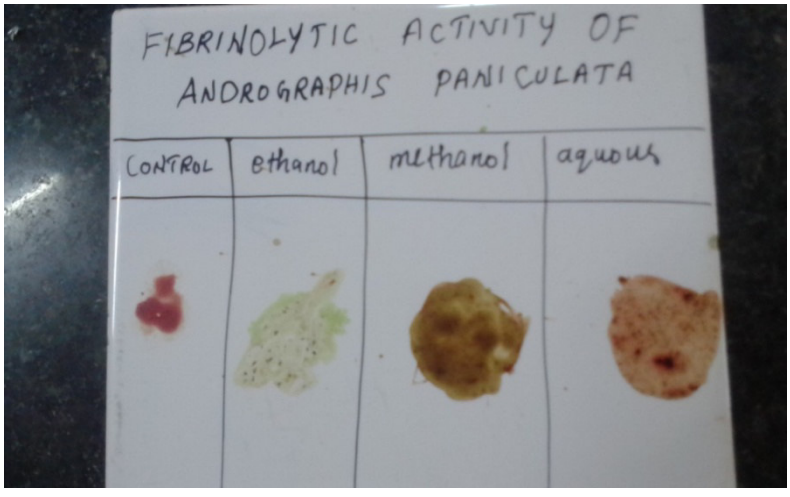
Figure 7
Anticoagulant activity of Andrographis paniculata



The result was observed in the figure 7, the anticoagulant activity of the aqueous extract showed there is no coagulation of blood, where as in ethanol and methanol *Andrographis paniculata* showed only mild anticoagulant activity. Anticoagulants (antithrombics, fibrinolytic, and thrombolytics) are a class of drugs that work to prevent the coagulation (clotting) of blood. A group of pharmaceuticals called anticoagulants can be used in vivo as a medication for thrombotic disorders. Some anticoagulants are used in medical equipment, such as test tubes, blood transfusion bags, and renal dialysis equipment[12.]

FIBRINOLYTIC ACTIVITY

Figure 8
Fibrinolytic activity of Andragraphis paniculata



The result as observed in the Figure 8, the fibrinolytic activity of the methanol and ethanol extract formed fibrin threads. This indicates that *Andrographis paniculata* has fibrinolytic activity whereas in aqueous extract also shows fibrinolytic activity. Fibrinolysis is a process that prevents blood clot[12]. In fibrinolysis its main enzyme plasmin cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver. Further research is needed to optimize the conditions and a new drug can be developed. In recent years focus on use of

non-traditional approaches to treat diseases has been revived worldwide. The evidence collected still now shows immense potential of medicinal plants used in traditional systems.

SUMMARY AND CONCLUSION

The selected medicinal plant are the source of the secondary metabolites i.e., saponins, phenols, tannins and steroids. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities of the medicinal plants are due to the presence of the above mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very helpful for the manufacturing of new drugs. The antibacterial efficacy of this plant confirms the claims of traditional healers who use to locate the active principles from various extract of the plants. The antioxidant activity of *Andrographis paniculata* (leaf) has moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that leaf can be used as a source of antioxidants for pharmacological preparations. This leaf also has the good fibrionlytic and anticoagulant activity. The medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies in the manufacturing of the new drugs for treatment of various diseases. Thus, we hope that the important phytochemical properties, antioxidant, antimicrobial, anticoagulant and fibrinolytic activity identified by our study in the local plant of *Andrographis paniculata* will be helpful in the copping different diseases of this particular region.

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