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Pankaj Kumar Umar
M.V.Sc Student, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Vidhi Gautam
Associate Professor, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Jyoti
Mvsc Student, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Shashank Vishvakarma
Mvsc Student, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Sachin Jain
Assistant Professor, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Corresponding Author:
Pankaj Kumar Umar
Mvsc Student, Department of Veterinary Pharmacology and Toxicology, COVS & A.H. (NDVSU), Jabalpur, Madhya Pradesh, India

Preliminary phytochemical analysis of *Andrographis paniculata*

Pankaj Kumar Umar, Vidhi Gautam, Jyoti, Shashank Vishvakarma and Sachin Jain

Abstract
Andrographis paniculata, commonly known as King of Bitters or Kalmegh, is an annual, branched, erect handsome herb running half to one meter in height and exhibits various therapeutics activities. The principle objective of the study was to access the phytochemical estimation of chemical constituents in *Andrographis paniculata*. The study involves preliminary phytochemical analysis of ethanolic extract of *Andrographis paniculata* which was screened for the presence of various phytoconstituents using standard procedure by soxhlet method, maceration method and incubator shaker method. The phytochemistry revealed the presence of phenol, flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, saponins, terpenoids, coumarins and quinones.

Keywords: Preliminary phytochemical, *Andrographis paniculata*, therapeutics activities

Introduction
Andrographis paniculata, commonly known as King of Bitters or Kalmegh, is an annual, branched, erect handsome herb running half to one meter in height. It exhibits anti-inflammatory, anti-HIV, antibacterial, antioxidant, antiparasitic, antispasmodic, antidiabetic, anticarcinogenic, antipyretic, hepatoprotective, nematocidal and various other therapeutics activities (Niranjan *et al*., 2010) [5]. Andrographolide and arabinogalactan proteins isolated from the *Andrographis paniculata* were screened for hepato-renal protective activity against ethanol-induced toxicity (Okhuarobo *et al*., 2014) [6].
Andrographolide is main active compound obtained from *Andrographis paniculata* and it has prominent role in hepatoprotection by reducing lipid peroxidation product malondialdehyde (MDA), as well as by maintaining high level of reduced glutathione, glutamic pyruvate transaminase and alkaline phosphatase. Andrographolide showed noticeable hepatoprotective effect in preventing carbon tetrachloride, paracetamol and galactosamine induced liver damage (Handa and Sharma, 1990) [2].
The objective of the present investigation was to screen the preliminary phytochemical analysis of *Andrographis paniculata* that contributes to various biological activities.

Materials and Methods
Collection and processing of plant
The whole plant of *Andrographis paniculata* was collected from Department of Plant Physiology, Jawaharlal Nehru Krishi Vishwa Vidyalaya (J.N.K.V.V.), Jabalpur (M.P.). Whole plant of *Andrographis paniculata* was processed for preparation of ethanolic extract by using soxhlet method (Thangaraj, 2017) [8], maceration method (Touaibia and Chaouch, 2016) and by incubator shaker method (Bahari *et al*., 2021) [1].

Preparation of ethanolic extract by soxhlet method
Crude extract was prepared by using ethanol (90 percent). About 20 gm of the coarsely grounded whole plant was taken in a thimble made up of whatman filter paper No. 1 and placed in soxhlet apparatus with 500 ml round bottom flask containing 400 ml solvent at a temperature 80±5 °C. The extraction was allowed to continue for 12 hrs. The extracts were collected in petri plates and kept in water bath at 90 °C for evaporating the extra solvent. The percent yield was calculated. The extract was kept in air tight container at 4 °C for further studies (Thangaraj, 2017) [8].

Preparation of ethanolic extract by maceration method

Whole plant was air-dried in shade at room temperature and powdered. 20 g of powdered plant material was macerated during 72 hours at room temperature with 200 ml ethanol (90 percent) under continuous magnetic stirring. The crude preparation was filtered through a 0.45 µm membrane filter. The filtered extract was then collected in petri plates and kept in water bath at 90 °C for evaporating the extra solvent. The percent yield was calculated. The extract was kept in air tight container at 4 °C for further studies (Touaibia and Chaouch, 2016) [9].

Preparation of ethanolic extract by cold maceration method in incubator shaker

The ethanolic extract of *Andrographis paniculata* was prepared as per the method given by Bahari *et al*. (2021) [1] with slight modification. Briefly, 10 percent concentration of extract was prepared by adding 20 gram of *Andrographis paniculata* dry powder into 200 ml of ethanol. The conical flasks were wrapped with cotton and aluminium foil and were transferred into an incubator shaker. They were kept at 37 °C for 24 hours with gentle shaking at 120 rpm. Then, the extract was filtered using whatman no. 1 filter paper in a filter funnel and the filtrate was collected. The filtrate was evaporated by using rotary evaporator at 70 °C. The concentrated extract was then transferred to glass petri dishes and dried in oven at 40 °C. The weight of extract was then measured after solvent evaporation and then kept into air tight containers.

Fig 1: *Andrographis paniculata*

Fig 2: Crude extract

Estimation of percent extractability

The percent extractability of ethanolic extract of *Andrographis paniculata* obtained by soxhlet method was calculated as described by Shukla, (2006) [7].

$$\text{Percent extractability} = \frac{\text{Weight of extract (g)}}{\text{Weight of the plant material (g) before extraction}} \times 100$$

Preliminary phytochemical analysis of plant extracts

The phytochemical analysis of ethanolic extract of *Andrographis paniculata* obtained by soxhlet method, maceration method and incubator shaker method were done as per the method described by Jeevalatha *et al.* (2022) [3]. The study was conducted as follows:

Test for Phenol Ferric chloride test

To 1 ml of the extract, 3 ml of distilled water was added followed by few drops of 10 percent aqueous ferric chloride solution. Formation of green colour indicates the presence of phenols.

Test for Flavonoids Shinoda test

To 2 ml of the extract, 1 ml of 1 percent ammonia solution was added. Appearance of yellow colour indicates the presence of flavonoids.

Test for Tannins Ferric chloride test

To 1 ml of the extract, 1 ml of 0.008 M potassium ferricyanide was added and then 1 ml of 0.02 M ferric chloride containing 0.1 N HCl was added. Appearance of blue–black colour indicates the presence of tannins.

Test for Alkaloids Mayer's test

Approximately, 1 ml of crude extract was mixed with 2 ml of wagner's reagent. Reddish brown colour precipitate indicates the presence of alkaloids.

Test for Carbohydrates Fehling's test

Equal volume of fehling A and fehling B reagents were mixed together and then add 2 ml of crude extract in it and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicates the presence of reducing sugars.

Test for Proteins

Millon's test

1 ml of crude extract was mixed with 2 ml of millon's reagent, white precipitate appeared which turned red upon gentle heating that confirmed the presence of protein.

a)

Ninhydrin test

1 ml of crude extract was mixed with 2 ml of 0.2 percent solution of ninhydrin and boiled. A violet colour precipitate was appeared suggesting the presence of amino acids and proteins.

Test for Glycosides

Extract was treated with 1 ml water and 1 ml sodium hydroxide. Formation of yellow colour indicates the presence of glycosides.

Test for Terpenoids Salkowski test

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated sulphuric acid was carefully added to form a

layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for Coumarin Coumarins test
10 percent sodium hydroxide was added to the extract and chloroform was added. Formation of yellow color shows the presence of coumarin.

Test for Saponins Foam test
2 ml of crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam is taken as an indication for the presence of saponins.

Test for Steroids Salkowski test
2 ml of acetic anhydride was added to 0.5 ml of crude extract containing 2 ml of sulphuric acid. The colour changed from violet to blue or green in samples indicates the presence of steroids.

Test for Quinones Quinone test
Diluted sodium hydroxide was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

Test for Anthraquinones Borntrager's test
0.5 g of each extract was boiled with 10 percent hydrochloric acid for few minutes in water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10 percent ammonia was added to the mixture and heated. Formation of rose–pink color indicates of the presence of the anthraquinones.

Result and Discussion
In the present study, powder of whole plant of *Andrographis paniculata* was extracted using ethanol solvent by three different extraction processes i.e. soxhlet method, maceration method and incubator shaker method. Ethanolic extract of *Andrographis paniculata* obtained by soxhlet method and maceration method was dark-green and brown-green respectively, in colour whereas ethanolic extract obtained by incubator shaker method was dry and light green in colour (Table 01).

Table 1: Colour and consistency of ethanolic extract of *Andrographis paniculata* obtained by different method of extraction

Name of the plant	Solvent used	Method of extraction	Colour of extract	Consistency of extract
Andrographis paniculate	Ethanol	Soxhlet	Dark green	Semisolid
	Ethanol	Maceration	Brown- green	Semisolid
	Ethanol	Incubator shaker	light green	Powder

Percent extractability of ethanolic extract of *Andrographis paniculata*
The percent extractability of ethanolic extract of *Andrographis paniculata* by soxhlet method, maceration method and incubator shaker method was calculated and depicted in Table 02.

Table 2: Percent extractability of ethanolic extract of *Andrographis paniculata*

Plant	Parts of the plant extracted	Solvent of extraction	Methods of extraction	Weight of the plant material (g) before extraction	Weight of extract (g)	Percent extractability
Andrographis paniculate	Whole plant	Ethanol	Soxhlet	20 g	4.0 g	20.00
			Maceration	20 g	4.6 g	23.00
			Incubator shaker	20 g	5.5 g	27.50

The highest percent extractability of *Andrographis paniculata* was obtained with incubator shaker method of extraction (27.50 percent) followed by maceration method of extraction (23.00 percent) and soxhlet method of extraction (20.00 percent).

Qualitative phytochemical screening
Ethanolic extract of *Andrographis paniculata* obtained by soxhlet, maceration and incubator shaker method were subjected for preliminary qualitative phytochemical screening to determine the presence of various active principles like phenol, flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, saponins, terpenoids, coumarins, quinones, steroids and anthraquinones.
Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by soxhlet method of extraction revealed the presence of phenol, flavonoids, tannins, alkaloids, carbohydrates, proteins, glycosides, saponins, terpenoids, coumarins and quinones (Table 03).
The findings of Umadevi and Kamalam (2014) [10] are in agreement with the findings of present study. They reported the presence of phenols, flavonoids, tannins, carbohydrates, proteins, terpenoids, alkaloids, glycosides, saponins, coumarins, quininones and steroids in ethanolic extract of *Andrographis paniculata* obtained by soxhlet method of extraction.

Table 3: Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by soxhlet method of extraction

S. No.	Phytochemicals	Qualitative Test	Observations and Result	
1.	Phenol	Ferric chloride test	Solution developed green colour	Present
2.	Flavonoids	Shinoda test	Solution developed yellow colour	Present
3.	Tannins	Ferric chloride test	Solution developed blue-black colour	Present
4.	Alkaloids	Mayer's test	Reddish brown colour precipitate developed	Present
5.	Carbohydrates	Fehling's test	Brick red precipitate developed	Present
6.	Proteins	Millon's test	Red precipitation developed	Present
		Ninhydrin test	Violet precipitation developed	Present
7.	Glycosides	Sodium hydroxide test	Solution developed yellow colour	Present
		Keller-Kiliani test	Solution developed violet and brown ring	Present
8.	Saponins	Foam test	Stable layer of foam developed	Present
9.	Terpenoids	Salkowski test	Solution developed Reddish brown colouration of interface	Present
10.	Coumarin	Coumarins test	Solution developed yellow colour	Present
11.	Steroids	Salkowski test	No specific colour developed	Absent
12.	Quinones	Quinone test	Solution developed blue green colour	Present
13.	Anthraquinones	Borntrager's test	No specific colour developed	Absent

Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by maceration method of extraction revealed the presence of phenol, flavonoids, tannins, alkaloids, carbohydrates, proteins, cardiac glycosides, saponins, terpenoids, coumarins and quinones (Table 04).

Table 4: Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by maceration method of extraction

S. No.	Phytochemicals	Qualitative Test	Observations and Result	
1.	Phenol	Ferric chloride test	Solution developed green colour	Present
2.	Flavonoids	Shinoda test	Solution developed yellow colour	Present
3.	Tannins	Ferric chloride test	Solution developed blue-black colour	Present
4.	Alkaloids	Mayer's test	Reddish brown colour precipitate developed	Present
5.	Carbohydrates	Fehling's test	Brick red precipitate developed	Present
6.	Proteins	Millon's test	Red precipitation developed	Present
		Ninhydrin test	Violet precipitation developed	Present
7.	Glycosides	Sodium hydroxide test	Solution developed yellow colour	Present
		Keller-Kiliani test	Solution developed violet and brown ring	Present
8.	Saponins	Foam test	Stable layer of foam developed	Present
9.	Terpenoids	Salkowski test	Solution developed reddish brown colouration of interface	Present
10.	Coumarin	Coumarins test	Solution developed yellow colour	Present
11.	Steroids	Salkowski test	No specific colour developed	Absent
12.	Quinones	Quinone test	Solution developed blue green colour	Present
13.	Anthraquinones	Borntrager's test	No specific colour developed	Absent

Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by incubator shaker method of extraction revealed the presence of phenol, flavonoids, tannins, alkaloids, carbohydrates, proteins, cardiac glycosides, saponins, terpenoids, coumarins, quinones and steroids (Table 05).

Malahubban (2013) [4] reported the presence of several active phytochemicals in ethanolic extract of *Andrographis paniculata* obtained by incubator shaker method. The extract revealed the presence of carbohydrates, steroids, fixed oils and fats, cardiac glycosides, tannins, flavonoids and saponins.

Table 5: Phytochemical screening of ethanolic extract of *Andrographis paniculata* obtained by incubator shaker method of extraction

S. No.	Phytochemicals	Qualitative Test	Observations and Result	
1.	Phenol	Ferric chloride test	Solution developed green colour	Present
2.	Flavonoids	Shinoda test	Solution developed yellow colour	Present
3.	Tannins	Ferric chloride test	Solution developed blue-black colour	Present
4.	Alkaloids	Mayer's test	Reddish brown colour precipitate developed	Present
5.	Carbohydrates	Fehling's test	Brick red precipitate developed	Present
6.	Proteins	Millon's test	Red precipitation developed	Present
		Ninhydrin test	Violet precipitation developed	Present
7.	Glycosides	Sodium hydroxide test	Solution developed yellow colour	Present
		Keller-Kiliani test	Solution developed violet and brown ring	Present
8.	Saponins	Foam test	Stable layer of foam developed	Present
9.	Terpenoids	Salkowski test	Solution developed reddish brown colouration of interface	Present
10.	Coumarin	Coumarins test	Solution developed yellow colour	Present
11.	Steroids	Salkowski test	Solution developed green colour	Present
12.	Quinones	Quinone test	Solution developed blue green colour	Present
13.	Anthraquinones	Borntrager's test	No specific colour developed	Absent

Conclusion

Preliminary phytochemical analysis of ethanolic extract of *Andrographis paniculata* obtained by Soxhlet, maceration and incubator shaker method revealed the presence of phenol, flavonoid, tannins, alkaloids, carbohydrate, glycoside, saponins, terpenoid, coumarin, quinones and confirmed the presence of diterpene compound, Andrographolide. According to the observational record of our present research work, it was found that highest per cent extractability of *Andrographis paniculata* was obtained with incubator shaker method of extraction (27.50 percent) followed by maceration method of extraction (23.00 percent) and soxhlet method of extraction (20.00 percent).

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