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Qualitative phytochemical analysis of leaf extracts of *Andrographis paniculata* and its antibacterial activity on poultry pathogens

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Abstract
The present work was undertaken to study the qualitative phytochemical analysis and testing of antibacterial activity of *Andrographis paniculata* aqueous and ethanolic leaf extracts against poultry pathogens. The leaf extracts of *Andrographis paniculata* were screened qualitatively for fourteen phytochemicals of which aqueous extract was found positive for saponins, tannins, alkaloids, terpenoids, flavonoids, hydrolysable tannins, glycosides and cardiac glycosides. Phenols, amino acids and protein, carbohydrates, phlobatannins, volatile oils and vitamin C were not detected. The ethanolic extract showed similar results to that of aqueous extract but glycosides could not be detected. Testing of antibacterial activity of the extracts on Muller Hinton agar plates showed 50 µL of aqueous leaf extract at 25 mg/ mL had maximum zone of inhibition (14mm) for *Pasteurella sp.* The activity of 50 µL of ethanolic leaf extract at 25 mg/ mL had maximum zone of inhibition for *Pasteurella sp.* (10mm) and *Staphylococcus sp.* (10mm) which is lower than the standard sensitive zone of inhibition and no zone of inhibition for other poultry pathogens such as *E.coli, Salmonella sp. and Klebsiella sp*.

Keywords: *Andrographis paniculata,* phytochemical analysis, antibacterial activity

Introduction
Andrographis paniculata is a member of the family of *Acanthaceae,* which has been used as a traditional herbal medicine in many parts of Asia and Europe (Jarukamjorn and Nemoto, 2008) [7]. It is known locally as Nilavembu, Sirunangai, Siriyanangai. The genus *Andrographis* consists of 28 species, only a few species are medicinal. It is an annual herb extremely bitter in taste. It is also known as Kalmegh or Kalamegha. It is commonly known as "king of bitters". Diterpenoids and flavonoids are the main chemical constituents of *A.paniculata* and these compounds are believed to be responsible for the biological activities of the plant (Tang and Eisenbrand, 1992) [18]. Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like Cancer (See *et.al.*, 2002, Sheeja *et.al.*, 2007 and Zhao *et.al.*, 2008) [14, 16, 20] and HIV infections (Calabrese *et.al.*, 2000) [4]. The plant shows antimalarial (Mishra *et al.*, 2009) [11], anti-inflammatory, antioxidant (Nees *et al.*, 2006) [13], antihepatitic, antihelmintic (Singh *et al.*, 2001) [17], antibacterial (Burm *et al.*, 2010) [3] antipyretic (Chandra *et al.*, 2010) and anticancer activity (Kumar *et al.*, 2004) [10]. Hence, the present study was carried out to study the qualitative phytochemical analysis and testing of antibacterial activity *Andrographis paniculata* leaf extracts against poultry pathogens.

Materials and Methods
Collection of *Andrographis paniculata* plant material
The leaves of *Andrographis paniculata* were collected from Erode district, Tamil Nadu through traditional herbal practitioners and authenticated by Botanical Survey of India, Coimbatore. The collected leaves were rinsed with distilled water, shade dried and powdered. The fine leaf powder was transferred into a sterile, air-tight container and used for extract preparation (Sharma and Joshi, 2011) [15].

Preparation of *Andrographis paniculata* leaf extracts
Ten per cent aqueous and ethanolic extracts of *A. paniculata* leaves were prepared by adding ten grams of dry powder to 100 mL of distilled water and 70 per cent alcohol, respectively. It was kept in a rotatory shaker for 48 hrs, filtered and then kept at 37 ºC for 48 hrs to evaporate the solvent. The dried material was stored in airtight container for further evaluation. Stock solution for antiviral and antibacterial activity was prepared by dissolving ten grams of dried

aqueous and ethanolic leaf extract each in 20 mL of phosphate buffered saline (PBS). Then serial dilution was made with PBS to prepare working solution (Nagajothi *et.al.*, 2018) [12].

Qualitative phytochemical analysis

Qualitative phytochemical analysis of aqueous and ethanolic extracts of *A. paniculata* was carried as per the method described by Harborne (1998) [6] at the laboratory of Ethno Veterinary Herbal Research Centre for Poultry, Veterinary Clinical Complex campus, VC&RI, Namakkal.

Antibacterial assay

Test organisms

The poultry pathogens *viz.*, *Pasteurella sp.*, *Staphylococcus sp.*, *E. coli*, *Salmonella sp.*, and *Klebsiella sp.*, collected from Poultry Disease Diagnosis and Surveillance Laboratory, TANUVAS, Namakkal was utilized for antibacterial assay.

Inoculum preparation

Inoculum of test organisms was prepared by growing pure isolates in Brain Heart Infusion Agar (BHIA) plates at 37 ºC for overnight. The colonies obtained from the plates were grown in Brain Heart Infusion Broth (BHIB) for three hours to obtain log phase culture. The organisms grown in BHIB was compared with 0.5 Mc Far land Standard to obtain 1.5 x 10^8 CFU/mL.

Assay of antibacterial activity using the agar well diffusion method

Agar-well diffusion method was used for determination of antibacterial activity (Baby Shalini and Sriman Narayanan, 2015) [2]. The dried aqueous and alcoholic leaf extracts of *Andrographis paniculata* were dissolved in phosphate buffered saline (pH 7.0) to the final concentration of 100 mg/mL and sterilized by filtration through 0.22 µm sterilized Millipore syringe filter. The agar plates were prepared by pour plate method using 20 mL Muller Hinton agar. The bacterial culture was suspended in PBS and diluted to 1.5 x 10^8 CFU/mL. The bacteria were streaked over the surface of Muller Hinton agar medium. Wells of 4 mm diameter were cut from the agar with a sterile borer and 50µL of aqueous and ethanolic extracts of different concentration *viz.*, 6.25, 12.5 and 25 mg/ mL was added to them. The inoculated plates were incubated at 37 ºC for 24 hrs. Antibacterial activity was evaluated by measuring the diameter of inhibition zone of the tested bacteria and compared with the standard antibiotic disc (Table 3) used for determining antibiotic sensitivity against the selected poultry pathogens.

Results and Discussion

Qualitative Phytochemical Screening

The result of phytochemical screening of aqueous and ethanolic extracts of *A. paniculata* is presented in Table 1.

Table 1: Qualitative phytochemical screening of aqueous and ethanolic extracts of *Andrographis paniculata*

S. No.	Phytochemicals	Aqueous extract	Ethanolic extract
1.	Saponin	+	+
2.	Tannin	+	+
3.	Phenol	-	-
4.	Alkaloids	+	+
5.	Terpenoids	+	+
6.	Flavonoids	+	+
7.	Aminoacids and protein	-	-
8.	Carbohydrates	-	-
9.	Phlobatannin	-	-
10.	Volatile oils	-	-
11.	Hydrolysable tannin	+	+
12.	Glycosides	+	-
13.	Cardiac glycosides	+	+
14.	Vitamin C	-	-

(+ Positive - Negative)

The extracts were screened for fourteen phytochemicals of which aqueous extract was found positive for saponins, tannins, alkaloids, terpenoids, flavonoids, hydrolysable tannins, glycosides and cardiac glycosides whereas phenols, amino acids and protein, carbohydrates, phlobatannins, volatile oils and vitamin C were not detected. The ethanolic extract showed similar results to that of aqueous but glycosides could not be detected in the ethanolic extract. The probable reason might be due to difference in extraction potential of the solvents (Kaur and Gupta, 2017) [8]. Both the extracts were positive for saponins, tannins, alkaloids, terpenoids, flavonoids, hydrolysable tannins, glycosides and cardiac glycosides which were earlier reported to be important for antiviral activity (Arbab *et al.*, 2017) [1]. In addition to the currently detected phytochemicals, the presence of quinones and steroids in *A. paniculata* was confirmed by Umadevi and Kamalam (2014) [19] and Nagajothi *et al.* (2018) [12].

Antibacterial activity of *Andrographis paniculata* leaf extracts

The antibacterial activity of the extracts on Muller Hinton agar plates with 50 µL of aqueous leaf extract with 25 mg/ mL showed maximum zone of inhibition (14 mm) for *Pasteurella sp.* and no zone of inhibition for other poultry pathogens. The activity of 50 µL of ethanolic leaf extract with 25 mg/ mL showed maximum zone of inhibition of 10 mm for *Pasteurella sp.* and *Staphylococcus sp.* and no zone of inhibition for other poultry pathogens (Table 2) which is lower than the standard sensitive zone of inhibition (Table 3). Baby Shalini and Sriman Narayanan (2015) [2] reported that methanol based leaf extract was best compared to other solvents used and 75 µL was optimum for all the test cultures and it was found to have more activity. Kanakavalli *et. al.* (2015) [9] also reported that methanol extracts of whole plant showed significant and highest antibacterial activity.

Table 2: Antibacterial activity of *Andrographis paniculata* aqueous and ethanolic leaf extracts against poultry pathogens

Extracts	Concentration (mg/mL)	Zone of inhibition(mm) of poultry pathogens				
		E. coli	*Salmonella*	*Pasteurella*	*Klebsiella*	*Staphylococcus*
Aqueous	25	-	-	14	-	-
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-
Ethanol	25	-	-	10	-	10
	12.5	-	-	-	-	-
	6.25	-	-	-	-	-

No zone of inhibition observed.

Table 3: Antibacterial activity of standard antibiotic discs against poultry pathogens

Antibiotic disc	Zone of inhibition(mm) of poultry pathogens				
	E.coli	*Salmonella*	*Pasteurella*	*Klebsiella*	*Staphylococcus*
COT 25	23-S	24- S	R	22- S	21- S
CTX 30	24-S	25-S	25-S	21 -S	18 -I
N 10	R	12	R	12	13
O 30	R	12	R	15	19
LE 5	R	22 -S	21 -R	18- R	18 -R

COT – Cotrimaxazole, CTX- Cefotaxime, N - Neomycin, O - Oxytetracycline, LE - Levofloxacin, S - Sensitive, I – Intermediate, R- Resistant

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