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

P Nithya, P Mekala and TR Gopalakrishnamurthy

DOI: https://doi.org/10.22271/phyto.2021.v10.i1aa.13634

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] and HIV infections (Calabrese et.al., 2000) [1]. The plant shows antimarial (Mishra et al., 2009) [11], anti-inflammatory, antioxidant (Nees et al., 2006) [13], antihepatic, 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 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.

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 (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.

**Results and Discussion**

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

| 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) [11]. 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].

**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.

**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                    | -        | -            | -             | -            | -               |
|          | 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- R        | R             | 22-S         | 21- S           |
| CTX 30          | 24-S     | 25- S        | 25- S         | 21 -S        | 18 -I           |
| N 10            | R        | 12 - R       | R             | 12           | 13              |
| O 30            | R        | 12 - R       | 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

Acknowledgement
The authors acknowledge the financial assistance and facilities provided by Tamil Nadu Veterinary and Animal Sciences University, Chennai-600051 to carry out the research.

References
1. Arbab AH, Parvez MK, Al-Dosari MS, Al-Rehaily AJ. *In vitro* evaluation of novel antiviral activities of 60 medicinal plants extracts against hepatitis B virus. Exp. Ther. Med 2017;14:626-634.
2. Baby Shalini V, Srimal Narayanan J. Antibacterial activity of *Andrographis paniculata* Nees against selective human pathogens. African Journal of Microbiology Research 2015;9(6):1122-1127.
3. Burn F, Kumar OA, Naidu LM, Rao KG. *In vitro* antibacterial activity in the extracts of Andrographis paniculata. International Journal of Pharm Tech Research 2010;2:1383-5.
4. Calabrese C, Berman SH, Babish JG, Ma X, Shinto L, Dorr M, et al. A phase I trial of andrographolide in HIV positive patients and normal volunteers. Phytotherapy Research 2000;14(5):333-8.
5. Chandra R, Kumarappan CT, Kumar J, Mandal SC. Antipyretic activity of JURU-01 - a polyherbal formulation. Global J. Pharmacol 2010;4(1):45-47.
6. Harborne AJ. Phytochemical methods: A guide to modern techniques of plant analysis. Springer Sci. Busi. Media 1998;2:4-16.
7. Jarukamjorn K, Nemoto N. Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. Journal of Health Science 2008;54(4):370-381.
8. Kaur N, Gupta J. Comparison of phytochemical extraction solvents for Andrographis paniculata. Res. J Pharm. Tech 2017;10(5):1271-1276.
9. Kanakavalli Kaniyan, Mohanapriya Hemalatha, Parthiban Thirumurthi, Thiruvanan, A review on antimicrobial herbs in Siddha medicine. International Journal of Pharmacognosy and Phytochemical Research 2015;7(1):72-75.
10. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Rajagopal S. Anticancer and immunostimulatory compounds from Andrographis paniculata. J Ethnopharmacol 2004;92:291-5.
11. Mishra K, Dash AP, Swain BK, Dey N. Anti-malarial activities of *Andrographis paniculata* and *Hedyotis corymbosa* extracts and their combination with curcumin 2009. [available at http://www.malariajournal.com/content/8/1/26] Malar J., 8:26.
12. Nagajothi S, Mekala P, Raja A, Raja MJ, Senthilkumar P. Andrographis paniculata: qualitative and quantitative phytochemical analysis. Journal of Pharmacognosy and Phytochemistry 2018;7(4):1251-1253.
13. Nees K, Sheeja PK, Shihab GK. Antioxidant and anti-inflammatory activities of the plant *Andrographis paniculata*. Immunopharmacol Immunotoxicol 2006;28:129-40.
14. See D, Mason S, Roshan R. Increased tumor necrosis factor alpha (TNF-alpha) and natural killer cell (NK) function using an integrative approach in late stage cancers. Immunol Invest 2002;31:137-153.
15. Sharma M, Joshi S. Comparison of anti-oxidant properties of *Andrographis paniculata* and *Tinospora cordifolia* leaves. J. Curr. Chem. Phar. Sc 2011;1(1):1-8.
16. Sheeja K, Guruvayoorappan C, Kuttan G. Antiangiogenic activity of *Andrographis paniculata* extract and andrographolide. Int. Immunopharmacol 2007;7:211-221.
17. Singh RP, Banerjee S, Rao AR. Modulatory influence of *Andrographis paniculata* on mouse hepatic and extrahepatic carcinogen metabolizing enzymes and antioxidant status. Phytother Res 2001;15:382-90.
18. Tang W, Eisenbrand G. Chinese Drugs of Plant Origin, Chemistry, Pharmacology and use in Traditional and Modern Medicine, Springer Verlag, Berlin 1992, 97-103.
19. Umadevi U, Kamalam M. Phytochemical and antioxidant studies on an important indigenous medicinal plant- *Andrographis paniculata* (Burm.f.) Nees. Int. J Pharm Sci Res 2014;5(12):5240-5244.
20. Zhao F, He EQ, Wang L, Liu K. Anti-tumor activities of andrographolide, a diterpene from *Andrographis paniculata*, by inducing apoptosis and inhibiting VEGF level. J Asian Nat Prod Res 2008;10:467-47.