



International Journal of Veterinary Sciences and Animal Husbandry



ISSN: 2456-2912

VET 2025; 10(2): 259-262

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www.veterinarypaper.com

Received: 06-11-2024

Accepted: 08-12-2024

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Phytochemical screening of *Andrographis paniculata*, garlic and turmeric as phytogenic feed additives used in poultry

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DOI: <https://doi.org/10.22271/veterinary.2025.v10.i2d.2086>

Abstract

Medicinal plants are widely used in treating various ailments and incorporated in the feed as phytogenic feed additives. The aim of this work was to explore the phytoconstituents in the leaves of *Andrographis paniculata*, turmeric rhizome and garlic bulb powder. The presence of phytochemicals in the plant products will provide an overview of pharmacological action on usage. Though many modern techniques are available to evaluate the phytochemicals in the extracts of phytogenic products, the qualitative tests are the preliminary method still used. The present study reports to have different classes of phytochemicals in all the extracts screened while saponin, amino acids and proteins, carbohydrates and vitamin C were absent.

Keywords: *A. paniculata*, aqueous extract, garlic, phytochemical screening, qualitative test, turmeric

Introduction

Phytogenic feed additives are those plant derived products (leaves, bark, root, flowers or buds, etc.) incorporated as feed additives in poultry diet which act as alternative to antibiotic growth promoters, improvement of digestibility, aids in nutrient absorption, immunomodulatory and maintaining the gut health (Athanasiadou *et al.* 2007) [2]. Medicinal plants are used in various applications since it is less toxic than synthetic chemicals, easily available and highly accessible. Medicinal plants contain active constituents called phytochemicals, the non-nutritive plant chemicals such as phenols, saponins, unsaturated lactones and cytogenic glycosides exhibiting protective or disease-preventive properties (Evans, 2009) [7]. Efficacy between phytogenic feed products has considerable variation in chemical composition due to climatic conditions, location, harvest stage or storage conditions, etc., (Applegate *et al.* 2010) [1]. During immune defense stress conditions, phytogenic feed additives aid to increase the intestinal availability of essential nutrients for absorption and thereby boosting the birds for better growth (Hashemi and Davoodi, 2010) [10]. Prabakar *et al.* (2016) [17] elucidated that plant based bioactive components (phytogenic feed additives) have been proven in poultry for digestion stimulating properties, growth promotion, antimicrobial activity, antioxidant and anti-stress property, gut microflora, immune enhancement and carcass meat safety and quality. *Andrographis paniculata* belongs to *Acanthaceae* family, a medicinal plant widely used in Ayurvedic preparations and in traditional system of medicine from time immemorial for various ailments to humans and animals. The plant is naturally available and cultivated in India, Srilanka, Mauritius and Pakistan. It is also named in various local names according to the Indian languages such as Kalmegh, Nilavembu, Kalupnath, Mahatila and Kiriati, etc. *Andrographis paniculata* or Kalmegh is called as “King of Bitters”. Garlic (*Allium sativum* L.) belongs to *Amaryllidaceae* family and native of central Asia. It is the most widely used plant product in dietary and for other medicinal applications (Fallah, 2015) [8]. Garlic has bioactive components like sulphur containing compounds (Alliin, diallylsulphides and allicin) which act as antibacterial, antifungal, antiparasite, antiviral, antioxidant, antithrombotic, anticancerous and vasodilator characteristics (Reuter *et al.*, 1996) [19].

Turmeric (*Curcuma longa*), a tropical herb of *Zingiberaceae* family, is widely used in indigenous medicine in Asia as an antimicrobial, endogenous stimulant, anti-flatulent and anti-inflammatory agent (Nouzarian *et al.* 2011) [15]. Medicinal properties are innumerable. The main constituent of turmeric is curcumin and other constituents present are volatile oils including tumerone, atlantone and zingiberone and sugars, proteins and resins (Leung 1980; Pawar *et al.*, 2014) [13, 16]. Furthermore, *Andrographis paniculata*, garlic and turmeric contain several metabolites with wide applications. The present study was conducted with the aim of preliminary qualitative analysis for the detection of phytochemicals present in the feed additives.

Materials and methods

Collection and authentication of plant materials

The leaves of *Andrographis paniculata* were obtained from the herbal garden located at Veterinary College and Research Institute, Orathanadu. The garlic bulbs and dry turmeric rhizomes were purchased from the local market. The taxonomical identification of the plant, turmeric rhizomes and garlic bulb were authenticated taxonomically by the Botanical Survey of India, Southern Regional Centre, Coimbatore, and Tamil Nadu.

Preparation of phytogenic feed additives

The leaves of *Andrographis paniculata* were shade dried, broken down into small pieces, then powdered using laboratory blender and sieved. The commercially available garlic bulbs and dry turmeric rhizomes were purchased from the local market and were shade dried. It was broken down into small pieces and powdered using laboratory blender and sieved. The powdered materials were stored in air tight containers separately. The phytogenic feed additives are depicted in Figure 1.

A. Extraction process

The aqueous extracts of *A. paniculata* leaf powder, turmeric rhizome powder and garlic bulb powder were prepared separately each with 10 g of powder in 100 ml of water and heated below 50°C and then kept at room temperature for cooling. Then it was filtered through filter paper and the extracts were kept at 4°C for further process and analysis.

B. Phytochemical analysis

The phytochemical analysis was carried out with the crude extracts obtained through extraction and were subjected to various tests using commonly employed precipitation and coloration reaction as per the methods of Harborne (1998) to reveal for the presence or absence of various phytoconstituents such as alkaloids, glycosides, cardiac glycosides, phenols, tannins, phlobatannins, hydrolysable tannins, flavonoids, terpenoids, saponins, amino acids and protein, carbohydrates, volatile oil and vitamin C.

1. **Test for Alkaloids:** To 2 ml of each extract in different test tubes, 2 ml of picric acid was added separately in each test tube and thus the formation of orange colour indicates the presence of alkaloids.
2. **Test for Glycosides:** In separate test tubes 2ml of each extract was taken and 2 ml of dilute sulphuric acid was added and then heated at 50°C for 2 minutes. Then 2 ml

of 10 per cent sodium hydroxide and 5 ml of Fehling's solution A and B were added. The formation of brick red indicates the presence of glycosides.

3. **Test for Cardiac glycosides:** To 2 ml of each extract in separate test tubes, 2 ml of glacial acetic acid, 1 drop of 10 per cent ferric chloride and 2 ml of concentrated sulphuric acid were added in a stepwise manner and kept aside without disturbing. The resulting formation of three layers of different colours i.e. upper layer-green colour, middle layer-brown colour and lower layer-violet colour indicates the presence of cardiac glycosides.
4. **Test for Phenols:** Each extract containing 2 ml in test tubes and 2 ml of 10 per cent ferric chloride solution was added and the formation of dark blue colour indicates the presence of phenols.
5. **Test for Tannin:** To 2 ml of each extract in test tubes, 2-3 drops of 10 per cent ferric chloride was added and the formation of blue or green colour indicates the presence of tannin.
6. **Test for phlobatannins:** The dilute hydrochloric acid of 1 ml was added to 2 ml of each extract in separate test tubes. The formation of red precipitate indicates the presence of phlobatannins.
7. **Hydrolysable tannin:** The aliquot 2 ml of each extract taken in separate test tubes were added with 2 ml of ammonia solution. The formation of emulsion like precipitate indicates the presence of hydrolysable tannin.
8. **Test for Flavonoids:** To the test tubes containing 2ml of each extract, few drops of 10 per cent sodium hydroxide was added. The formation of yellow colour and its disappearance by addition of 1 ml of dilute hydrochloric acid along the sides of the test tube indicates the presence of flavonoids.
9. **Test for Terpenoids:** To 2 ml of each extract in separate test tubes, 2 ml of chloroform was added. Then along the sides of the test tube, 2 ml of concentrated sulphuric acid was added. The formation of brown colour ring at the junction of two liquids indicates the presence of terpenoids.
10. **Test for saponins:** To 2 ml of each extract, 10 ml of distilled water was added and then shaken for 10 minutes. The formation of layer of foam which remains for 10 minutes indicates the presence of saponin.
11. **Test for amino acids and protein:** To 2 ml of each extract in different test tubes, 1 ml of ninhydrin solution was added and then heated under flame at 50°C for 1 minute. The formation of violet colour indicates the presence of protein.
12. **Test for carbohydrates:** To 2 ml of each extract in test tubes, 2 ml of Fehling's solution of A and B was added and then heated at 50°C for 1 minute. The brick red precipitation formation indicates the presence of carbohydrates.
13. **Volatile oil:** With 2 ml of each extract in separate test tubes, 0.1 ml of sodium hydroxide solution and few drops of dilute hydrochloric acid were added. The formation of white precipitate indicates the presence of volatile oil.
14. **Vitamin C:** In test tubes 2 ml of each extract was added and mixed with 2 ml of sodium bicarbonate solution. The formation of violet colour indicates the presence of vitamin C.



Fig 1: Photograph shows the raw and powdered phytogetic feed additives

Results and Discussion

The results of the phytochemical screening of aqueous extract of *A. paniculata* leaves, garlic and turmeric are presented in Table 1. The phytochemical analysis of aqueous extract of *A. paniculata* leaves revealed the presence of alkaloids, tannin, phlobatannin, terpenoids and flavonoids. The qualitative phytochemical analysis of aqueous extract of *A. paniculata* leaves revealed higher flavonoid content but lower phenol levels which is in accordance with the findings of Dwivedi *et al.* (2015) [6] reported that the phytochemical constituents in the dried leaves of *Andrographis paniculata* subjected to three solvents viz., petroleum ether, methanol and water revealed the presence of alkaloids, steroids, flavonoids, tannins, triterpenoids, quinones and protein in different solvent extracts. Rajalakshmi and Cathrine (2016) [18] reported that the ethanolic extract of *Andrographis paniculata* leaves for

phytochemicals analysis revealed the presence of alkaloids, carbohydrates, saponin, protein, phytosterol, phenolic compounds, flavonoid and glycoside. Nagajothi *et al.* (2018) [14] reported that phytochemicals in aqueous and ethanolic extracts of *Andrographis paniculata* leaves found alkaloids, phenols, tannins, phlobatannins, hydrolysable tannins, flavonoids, terpenoids and saponins in aqueous extract, whereas ethanolic extract had similar phytochemicals and also positive for cardiac glycosides except for phlobatannins. Junaid and Patil (2020) [12] reported that the medicinal utilities are determined by the active principles present in the plant and the biological activities are exhibited through the phytochemicals available in it. The phytochemical analysis reveals the presence or absence of phytochemicals in the plant substances.

Table 1: Qualitative phytochemical analysis of aqueous extract of *A. paniculata* leaves, garlic and Turmeric

S. No.	Phytochemicals	<i>Andrographis paniculata</i>	Garlic	Turmeric
1.	Alkaloids	++	++	=
2.	Saponin	=	=	=
3.	Tannin	++	=	=
4.	Phenol	=	=	=
5.	Glycosides	=	=	=
6.	Phlobatannin	+	=	+
7.	Carbohydrates	=	=	=
8.	Hydrolysable tannin	=	++	+
9.	Terpenoids	++	++	++
10.	Flavonoids	++	++	++
11.	Protein and amino acids	=	=	=
12.	Volatile Oil	=	++	++
13.	Cardiac glycosides	=	++	++
14.	Vitamin C	=	=	=

The aqueous extract of garlic powder on phytochemical analysis revealed the presence of alkaloids, hydrolysable tannin, terpenoids, flavonoids, volatile oils and cardiac glycosides. The results of this study are in concurrence with Huzaifa *et al.* (2014) ^[11], Divya *et al.* (2017) ^[5] who reported that qualitative phytochemical screening of aqueous extract of garlic (*Allium sativum*) bulbs using the standard method of analysis showed the presence of flavonoids, alkaloids, saponins, tannins and cardiac glycosides.

The phytochemical screening of aqueous extract of turmeric revealed the presence of phlobatannin, hydrolysable tannin, terpenoids, flavonoids, volatile oil and cardiac glycosides. The results of this study are in accordance with the findings of Deb *et al.* (2013) ^[3] that aqueous extract contains alkaloids, amino acids and flavonoids whereas ethanolic extract has flavonoids, tannins and amino acids whereas Pawar *et al.* (2014) ^[16] reported that phytochemical analysis in turmeric rhizomes elucidated that 95 per cent ethanol crude extract of turmeric indicated the presence of carbohydrates, proteins, amino acids, alkaloids, terpenoids and flavonoids.

Conclusion

The qualitative phytochemical analysis of aqueous extract of *A. paniculata* leaves, garlic and turmeric revealed the presence of various phytochemical compounds. Hence, from this study the phytochemical analysis helps to determine the bioactive compounds presence or absence in it for further application as phytochemical feed additive in the poultry diet. Further, the phytoconstituents present in the plant may varied according to the stages and cultivation of the plant and differs based on the extraction methods.

Acknowledgement

The authors acknowledge the funding and the other facilities required for the study was provided by Tamil Nadu Veterinary and Animal Sciences University, Chennai throughout the study.

Conflict of Interest: Not available

Financial Support: Not available

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How to Cite This Article

Mehala C, Kannan D, Ramachandran M. Phytochemical screening of *Andrographis paniculata*, garlic and turmeric as phytochemical feed additives used in poultry. *International Journal of Veterinary Sciences and Animal Husbandry.* 2025;10(2):259-262.

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