Phytochemical analysis and biological activities of *Andrographis paniculata*

Abstract

Introduction: Medicinal plants serve as a crucial reservoir of natural bioactive molecules, widely harnessed in traditional therapeutic systems. Their ability to modulate the immune system plays a key role in maintaining homeostatic balance. Herbal remedies are also known to contribute to improved health outcomes and are often used to delay the onset of degenerative and age-associated ailments. Among these, *Andrographis paniculata* (Burm.f.) Nees, also known as the "King of Bitters", is a well-known medicinal herb native to South and Southeast Asia, including India, China, and Thailand, *etc*. It has a long-standing use in ethnomedicine, particularly for treating inflammatory disorders and dermatological infections.
Aims: The current investigation focused on analyzing the phytochemical constituents of *Andrographis paniculata* leaf extract prepared using water solvent (APAE). Preliminary phytochemical screening and Gas Chromatography-Mass Spectrometry (GC-MS) of APAE were employed to profile its chemical composition. Additionally, the extract's biological properties, immunomodulatory activities, were assessed *in vitro* using chicken lymphocytes culture.
Results: APAE confirmed the presence of different tested classes of phytochemicals, including carbohydrates, tannins, saponins, flavonoids, alkaloids, *etc*. GC-MS profiling of APAE revealed 12 bioactive compounds in the extract. The Maximum Non-Cytotoxic Dose (MNCD) of the extract in cultured chicken lymphocytes was determined via MTT assay and found to be 150 µg/mL. In lymphocytes proliferation assay (LPA), APAE revealed significant immunomodulatory property under mitogenic stimulation.
Conclusions: These findings highlight the potential of *Andrographis paniculata* as a natural source for developing effective immunomodulatory agents. However, elaborate *in vitro*/ *in vivo* studies are needed to exploit this bioresource further for developing immunomodulatory preparation for specific usage.

1. INTRODUCTION

Medicinal plants represent a vital source of biologically active substances that are integral to both traditional healing systems and contemporary medical practices. These plant-derived compounds are widely employed in developing nutraceuticals, health supplements, folk treatments, and as foundational materials for pharmaceutical formulations and synthetic drug design (Ekor, 2014; Ambwani *et al*., 2018). The health-promoting and immunomulating effects of these botanicals are largely attributed to their rich phytochemical profiles, which help in defending the body against environmental insults and maintaining internal homeostasis (Ambwani *et al*., 2019; Pandey and Ambwani, 2022; Ambwani *et al*., 2025). Beyond therapeutic applications, many herbal formulations are consumed as dietary supplements aimed at promoting general well-being and reducing the risk of chronic, age-associated conditions (Yadav *et al*, 2016). Oxidation is a process where electrons are transferred from one atom to another, with the molecule losing an electron being oxidized. Free radicals are generated when oxidation occurs during aerobic respiration. Fortunately, there are natural defense mechanisms to reduce the damage done by free radicals. Antioxidants are the main defense mechanism in the body acting as free radical scavengers. Regardless of the sources of antioxidants, all the antioxidants have a similar function, which is to prevent damage done by free radicals. In recent years, attention has grown around the antioxidant capabilities of medicinal plants, particularly for their role in reducing oxidative stress and preventing tissue injury induced by free radicals. A wide range of botanicals has been explored for their potential antioxidant properties in this context (Upadhyaya *et al*., 2011).

Andrographis paniculata (Burm.f.) Nees, a prominent member of the Acanthaceae family, is commonly referred to as ‘Kalmegh’ in India and widely known across Asia as the ‘King of Bitters’ (Kaushik *et al*., 2021). This herbaceous plant is indigenous to the Indian subcontinent, particularly India and Sri Lanka, and has now spread across various parts of Asia, including China, Vietnam, Thailand, Cambodia, Laos, Malaysia, Indonesia, Taiwan, and even the Caribbean islands (Jiao *et al*., 2019). Traditionally, *Andrographis paniculata* has been employed in treating a wide spectrum of ailments such as fevers (including dengue and malaria), insect bites, snake envenomation, diarrheal diseases, respiratory tract infections, and dermatological conditions (Sujarwo *et al*., 2015; Jiao *et al*., 2019; Nayak *et al*., 2020).

Its therapeutic usage is firmly rooted in several traditional medicinal systems, notably Ayurveda, Siddha, Unani, and naturopathy in India, as well as Traditional Chinese Medicine (TCM) and Thai herbal practices. The plant’s extracts are known to contain bioactive compounds that contribute to a diverse array of pharmacological effects. Scientific investigations have documented its properties, which include analgesic, anticancer, antidiabetic, antifertility, anti-inflammatory, antimalarial, antimicrobial, antioxidant, antipyretic, antiviral (including antiretroviral), antivenom, cardioprotective, hepatoprotective, and immunomodulatory activities (Singha *et al*., 2003; Dai *et al*., 2019; Kaushik *et al*., 2021; Wong *et al*., 2021).

Phytochemical investigations have shown that *Andrographis paniculata* contains a wide array of bioactive constituents. Among these, terpenoid lactones and flavonoids are the predominant chemical classes. Additionally, compounds such as di- and triterpenoids, phenolic acids, xanthones, and various volatile constituents have also been identified in different parts of the plant (Gupta *et al*., 1983; Rao *et al*., 2004; Chandra *et al*., 2016; Kumar *et al*., 2018). Globally, more than 80% of the population relies on herbal medicines, either directly or indirectly, due to their perceived safety and minimal side effects. This growing interest has

emphasized the need for ensuring the quality and authenticity of both raw herbal materials and finished formulations. This study was conducted to assess the immunomodulatory potential of water extract of aerial parts of *Andrographis paniculata* (APAE) utilizing the chicken lymphocytes. APAE was also analyzed for the presence of various phytochemicals through biochemical assays and GC-MS analysis.

2. MATERIALS AND METHODS

Collection of plant material and Preparation of Plant Extract

Fresh aerial parts- stem and leaves of *Andrographis paniculata* were collected from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar, Uttarakhand, India. After washing thoroughly, the collected plant material was shed dried and powdered. The dried powder was subjected to extraction using an aqueous solvent system, following established protocols reported in earlier studies (Thakur *et al*, 2018a; b). To maintain experimental consistency and minimize contamination, all reagents and solvents employed were of animal cell culture grade.

Qualitative Biochemical analysis of APAE

APAE was subjected to different phytochemical tests as per the methods described by Thakur *et al*. (2018a; b; c) to test the presence of proteins, carbohydrate, alkaloids, tannins, flavonoids, steroids and saponins.

Gas Chromatography–Mass Spectrometry (GC-MS) analysis

GC-MS analysis was out sourced from Jawaharlal Nehru University (JNU), New Delhi, India Shimadzu QP2010 instrument equipped with a split-less injector and a mass selective detector, along with a suitable capillary column was utilized. For the analysis of the plant extract (APAE), the following conditions were maintained: the column oven temperature was set at 100 °C, with a pressure of 175.1 kPa. The system operated at a total flow rate of 16.3 mL/min, a column flow of 1.21 mL/min, a linear velocity of 28.9 cm/sec, and a purge flow of 3.0 mL/min. The mass detector recorded data starting at 6.00 min and ending at 40.49 min. Compounds were identified by comparing their mass spectra with those available in the NIST and WILEY spectral libraries. The name, molecular weight, and structural characteristics of each component were determined based on this spectral matching.

In vitro antioxidant ability of APAE

To assess the antioxidant potential of APAE, hydrogen peroxide scavenging method was used as described by Ruch *et al.* (1984).

Assessment of *In Vitro* Immunomodulatory Activity of APAE

To assess the biological activity of *Andrographis paniculata* aqueous extract (APAE)- first its suitable cell culture dose was established using MTT assay and the immunomodulatory potential was evaluated using lymphocytes proliferation assay under mitogenic stimulation.

Isolation of Chicken Lymphocytes

Peripheral blood was collected from healthy broiler chickens aged 4–6 weeks, sourced from a local poultry processing unit. The samples were transported to the laboratory under sterile conditions. Lymphocytes were isolated using a standard density gradient centrifugation technique under aseptic conditions. Cell viability exceeding 95% was verified using the trypan blue dye exclusion method, confirming the high integrity of the isolated cells.

Determination of Maximum Non-Cytotoxic Dose (MNCD) and Lymphocyte Proliferation Assay (LPA)

The cytotoxicity of APAE was evaluated using the MTT assay, which assesses mitochondrial enzymatic activity as an indicator of cell viability (Kakade *et al*., 2018; Ambwani *et al*., 2024; Thakur *et al*., 2025). Isolated lymphocytes were seeded into 96-well flat-bottom microplates at a concentration of 1×10^6 cells/mL. Cells were treated with serial concentrations of APAE ranging from 0.001 to 1.0 mg/mL and incubated for 68 hours at 40°C in a humidified incubator containing 5% CO_2. After incubation, 20 µL of MTT reagent (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 hours in the dark. The resulting purple formazan crystals were solubilized using 200 µL of DMSO per well, and absorbance was measured at 570 nm using an ELISA plate reader. Cell viability was calculated as a percentage relative to untreated controls. To evaluate the immunostimulatory effect of APAE, lymphocyte proliferation assay was conducted according to standard methods (Kakade *et al*., 2018; Ambwani *et al*., 2024; Thakur *et al*., 2025). Lymphocytes (1×10^6 cells/mL) were plated in 96-well plates with or without the addition of mitogens. The mitogens used were Concanavalin A (ConA) and lipopolysaccharide (LPS) from *E. coli*, each applied at a concentration of 5 µg/mL. The MNCD of APAE, as determined from the MTT assay, was added to the respective test wells. All treatments were performed in triplicate to ensure experimental repeatability and statistical robustness.

Statistical Analysis

The experimental data were analyzed using one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) post hoc test for multiple comparisons between treated and control groups. Results were expressed as mean values with their corresponding standard errors (± SE). Pearson correlation coefficients were calculated to assess relationships among variables. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS software.

3. RESULTS

***Andrographis paniculata* Extract**

The aqueous extract of *Andrographis paniculata* (APAE) was prepared with the percent yield of 17.74% (Table 1; Figure 1).

Table 1: Per cent Yield of APAE

Medicinal plant extract	Dry weight (gram)	Final weight of extract (gram)	Per cent yield
Aqueous extract of *Andrographis paniculata* (APAE)	50	8.87	17.74

A. *Andrographis paniculata*

B. Dried aerial part powder of *Andrographis paniculata*

C. Lyophilized aqueous extract of *Andrographis paniculata* (APAE)

Figure 1: Plant material and plant extract

Phytochemical analyses of APAE

Biochemical screening of APAE confirmed the presence of different tested classes of phytochemicals, including carbohydrates, tannins, saponins, flavonoids, alkaloids, steroids, phenols, and glycosides (Table 2).

Table 2: Qualitative Phytochemicals found in APAE

Biochemical Tests	APAE
Test for protein	+
Test for carbohydrates	+
Test for resins	+
Test for tannins	+
Test for Sapomims	+
Test for flavonoids	+
Test for alkaloids	+
Test for steroids	+
Test for phenols	+
Test for glycosides	+

GC-MS Analysis of APAE

GC-MS analysis of APAE showed a total of 12 phytochemicals as presented in Table 3 and Figure 2.

Table 3: Phyto-constituents present in APAE after determining the retention time peaks with NYST AND WELLEY Library.

Peak #	R. Time	Area %	Name	Mol. formula	CAS No	Mol. Wt.
1	7.449	31.14	2-Ethyl-3-methyl-1-butene	C_7H_{14}	7357-93-9	98
2	10.263	0.62	Hexane	C_6H_{14}	110-54-3	86
3	10.557	1.04	Lauric acid ME	$C_{13}H_{26}O_2$	111-82-0	214
4	11.063	1.33	Myristic acid	$C_{14}H_{28}O_2$	544-63-8	228
5	11.834	0.51	4-Nonenoic acid, Methyl ester, Methyl (4e)-4-nonenoate	$C_{10}H_{18}O_2$	20731-19-5	170
6	11.95	19.77	Palmitic acid ME	$C_{17}H_{34}O_2$	112-39-0	270
7	12.394	4.76	9-Octadecenoic acid (Z)-, Octadec-9-enoic acid, (9e)-9-	$C_{18}H_{34}O_2$	112-80-1	282

			octadecenoic acid			
8	13.338	29.11	Brassidic acid ME	$C_{18}H_{32}O_2$	60-33-3	280
9	13.518	3.64	Eicosanoic acid ME	$C_{21}H_{42}O_2$	1120-28-1	326
10	13.874	7.1	Cyclotetradecane	$C_{14}H_{28}$	295-17-0	196
11	16.193	0.57	Cyclohexanone	$C_6H_{10}O$	108-94-1	98
12	18.197	0.4	Diisooctylphthalate	$C_{24}H_{38}O_4$	27554-26-3	390

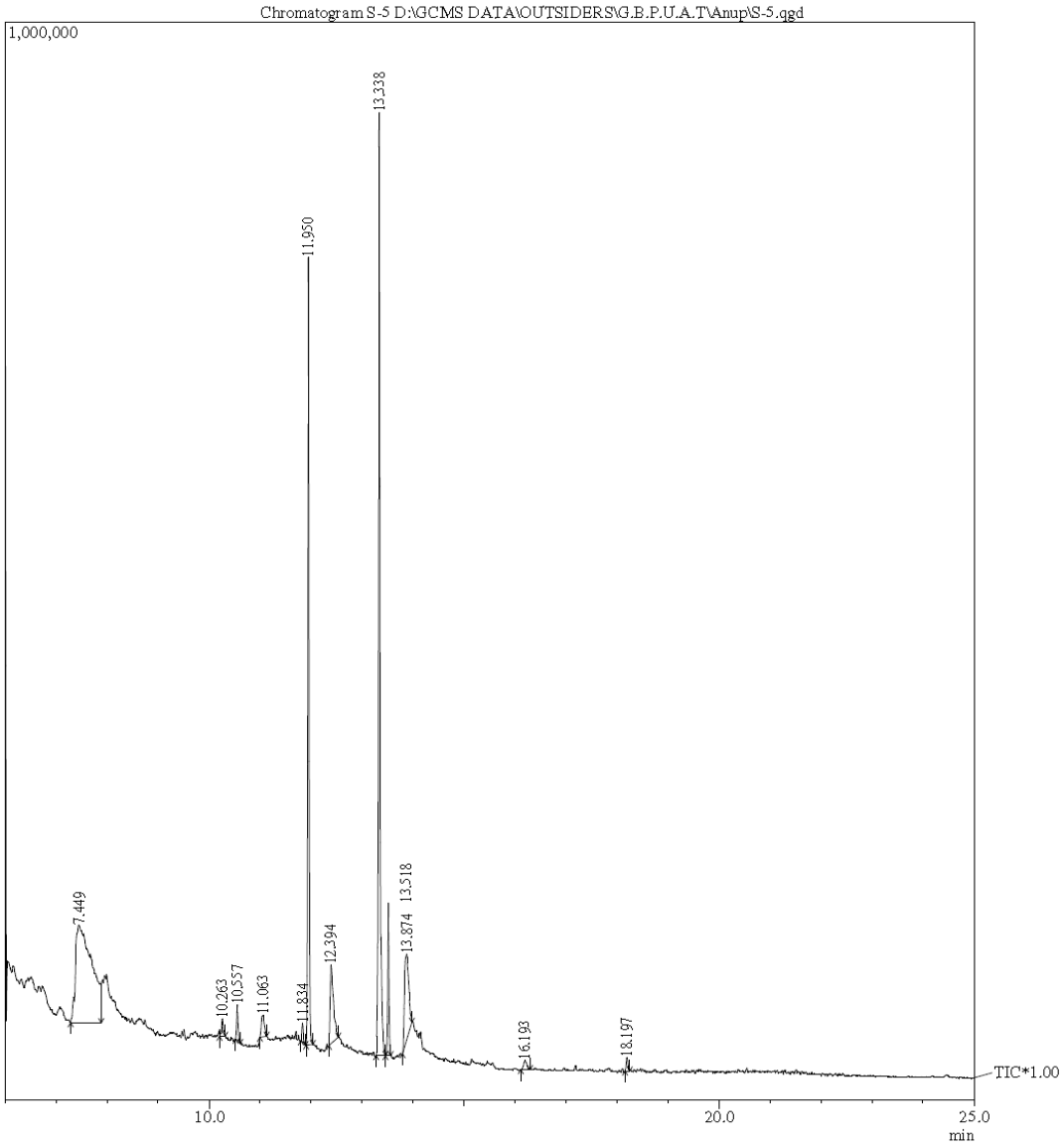

Figure 2: Chromatogram showing peaks for phyto-constituents in APAE

Hydrogen peroxide scavenging effects of APAE

To assess the antioxidant potential of APAE, hydrogen peroxide scavenging method was used. The per cent H_2O_2 scavenging activity of *Andrographis paniculata* was 20.13% as compared to control.

Non-cytotoxic dose of *Andrographis paniculata* in chicken lymphocytes

The avian lymphocytes were exposed to various dilutions of APAE to determine its maximum non-cytotoxic dose for further *in vitro* studies. The data indicated dose-dependent cytotoxicity induced by APAE lymphocytes culture. At higher concentrations of APAE *i.e.*, 0.5 mg/ml and 1 mg/ml, more was the cytotoxicity with maximum cytotoxicity of 19.98% at the highest concentration of 1 mg/mL used in the study. Concentrations of APAE ranging from 0.3mg/ml to 0.001mg/ml showed 100% cell viability (Table 4; Figure 3). Since maximum concentration of APAE that showed 100% cell viability was 0.3mg/ml, this was selected for further *in vitro* analysis.

Table 4: Non-cytotoxic dose of APAE by MTT assay

Concentration of APAE	**% Viability rate**	**% Cytotoxicity**
1.0 mg/ml	80.010	19.989
0.9 mg/ml	90.885	9.115
0.8 mg/ml	91.791	8.209
0.7 mg/ml	91.403	8.596
0.6 mg/ml	93.219	6.784
0.5 mg/ml	94.251	5.748
0.4 mg/ml	99.17	0.832
0.3 mg/ml	100.0	0
0.2 mg/ml	100.0	0
0.1 mg/ml	100.0	0
0.05 mg/ml	100.0	0
0.001 mg/ml	100.0	0
0.0005 mg/ml	100.0	0
0.0001 mg/ml	100.0	0
Cd at 1% 0.033612	**Cd at 5%** 0.02517	**3.594827** (highly significant)**

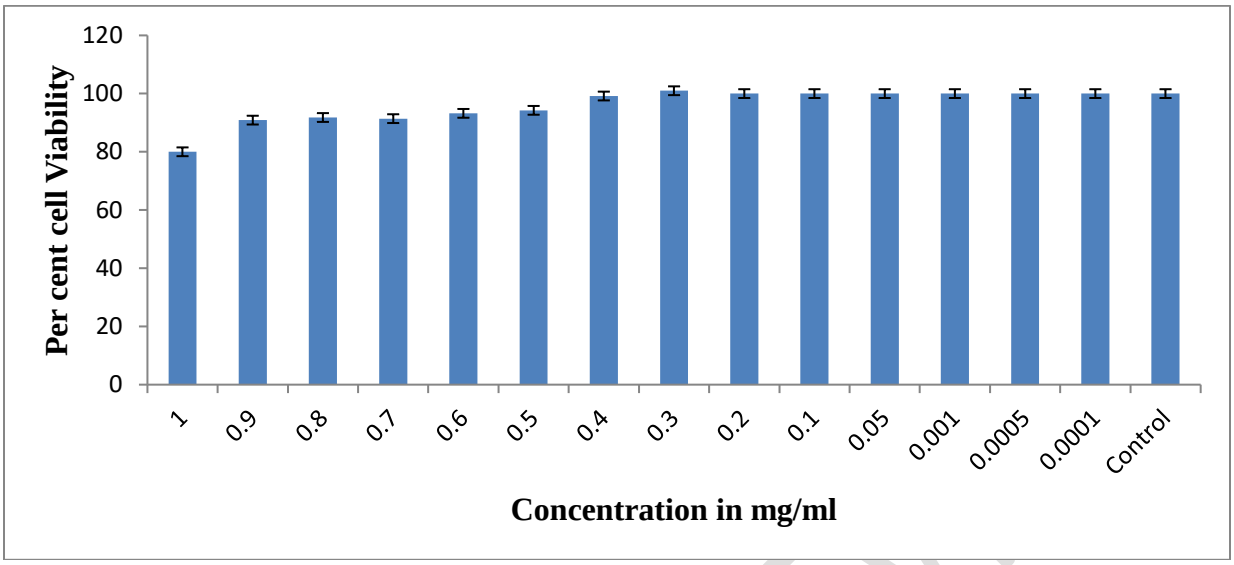

Figure 3: Non-cytotoxic dose of aqueous extract of *Andrographis paniculata* by MTT cytotoxicity assay

In vitro* Immunomodulatory activity of *Andrographis paniculata

As compared to untreated control, APAE displayed 15.91% more proliferation in chicken lymphocytes. There was marked increase of 4.89% in B cell blastogenesis in case of APAE treated cells as compared to control, whereas blastogenic capacity of T cells was significantly suppressed by APAE and demonstrated viability up to 96.51% as compared to control (Table 5).

Table 5: Per cent change in proliferation of lymphocytes due to *in vitro* exposure of APAE

Treatment	Unstimulated		LPS stimulated		ConA stimulated	
	% Change in proliferation	% change in proliferative index	% Change in proliferation	% change in proliferative index	% Change in proliferation	% change in proliferative index
Control	100	0	100	0	100	0
APAE	115.918	15.91	104.899	4.899	96.512	-8.14
Cd value at 1 %	0.073505	4.133039*	0.088607	4.751738*	0.059765	12.08968**
Cd value at 5 %	0.052453		0.063229		0.0426478	

4. DISCUSSION

In Indian traditional medicine, *Andrographis paniculata* is widely used in multiple forms—such as decoctions, juices, pastes, powders, and extracts prepared from the leaves, aerial parts, roots, or the entire plant—to manage a variety of ailments (Mishra *et al*., 2007). It is commonly prescribed for different types of fevers, including chronic, malarial, typhoid, and heat-induced fevers (Tiwari & Yadav, 2003; Sivasankari *et al*., 2014; Murtem & Chaudhry, 2016). It is traditionally employed in treating respiratory tract infections like influenza, whooping cough, bronchitis, and asthma (Amroyan *et al*., 1999; Ayyanar & Ignacimuthu, 2005). Its applications extend to dermatological disorders—such as eczema, leprosy, skin allergies, scabies, itches, and

warts—and parasitic infections, including intestinal worms and ringworm (Ayyanar & Ignacimuthu, 2005). This plant is also recognized for its ethnomedicinal use in neutralizing venom from snakebites and scorpion stings (Sivasankari at el., 2014; Silambarasan & Ayyanar, 2015; Nyeem *et al*., 2017). *Andrographis paniculata* has also been traditionally used in the management of musculoskeletal conditions like joint pain, gout, arthritis, and frozen joints (Hossain *et al*., 2021). It has also been used in traditional settings for managing syphilis, HIV/AIDS, cancer, and headaches. The herb is valued for its roles as a blood purifier, antipyretic, anthelmintic, and general tonic for debility and weakness (Ayyanar & Ignacimuthu, 2005; Dai *et al*., 2019).

The current study aimed to evaluate the phytochemical composition and biological activity of *Andrographis paniculata* extract, particularly focusing on its immunomodulatory properties. The antioxidant potential of the extract can be attributed to the presence of diverse secondary metabolites such as terpenoids, flavonoids, and sterols, which are well-documented for their capacity to neutralize free radicals (Owoade *et al*., 2021). Moreover GC-MS analysis exhibited presence of 12 phytochemicals. Oxidative stress has been implicated in the pathogenesis of several immune and inflammatory disorders; therefore, natural compounds with antioxidant activity can play a supportive role in immunoregulation. Furthermore, the extract demonstrated significant immunomodulatory effects in chicken lymphocytes. Atwijukire *et al* (2025) reported immunomodulatory activity of *Andrographis paniculata* in managing Plasmodium infection. Anti-inflammatory activity of compounds from Andrographis paniculata was reported (Chao *et al*., 2010; Gan *et al*., 2019). In one of the studies andrographolide was reported to reduce IFN-γ and IL-2 production in murine T-cells stimulated with concanavaline A (Con A) *in vitro* (Burgos *et al*., 2020). Various studies demonstrated the immunomodulatory potential of *Andrographis paniculata* (Chandewar *et al*., 2016; Gan *et al*., 2019; Lee *et al*., 2020). The observed MNCD of 150 µg/mL for the extract ensures safety in vitro and provides a baseline for further dose-optimization studies. The study contributes valuable data on the immunopharmacological profile of *Andrographis paniculata*, which may be leveraged for the development of plant-based immunotherapeutic or nutraceutical products.

5. CONCLUSION

This study demonstrates that the aqueous aerial extract of *Andrographis paniculata* contains a diverse range of bioactive compounds. The extract exhibits potent immunomodulatory activities *in vitro*, without causing cytotoxicity up to a concentration of 150 µg/mL in chicken lymphocytes cultures. These findings highlight the potential of *Andrographis paniculata* as a natural source for developing safe and effective immunomodulatory agents. Further studies, particularly *in vivo* trials and clinical validations, are warranted to confirm its therapeutic efficacy and safety profile.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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