Sys Rev Pharm 2024; 15(12): 373-376
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453/DOI: 10.31858/0975-8453.15.12.373-376

Research Article

In vitro Antioxidant Potential of *Andrographis paniculata* Extracts

Onyeneke Eusebius Chukwu, Boluwade Oluwaropo*, Victor Omozuwa, Chibuike A. Onwe, Olowoyo Martins Omorogie, Emmanuel Onwordi

Department of Biochemistry, University of Benin, Benin city, Nigeria

Article History: *Submitted: 03.12.2024* *Accepted: 23.12.2024* *Published: 31.12.2024*

ABSTRACT

Andrographis paniculata is a well-known herbaceous plant with numerous therapeutic properties. It has been utilized in traditional medicine to treat and manage various pathological conditions, including high blood pressure, diabetes, cancer, malaria and cough, across Asia, America and Africa. The therapeutic effects of *Andrographis paniculata* are attributed to the presence of several bioactive compounds within the plant.

This study aimed to investigate the antioxidant potentials of different extracts of *Andrographis paniculata*, specifically aqueous, carbonated drink and ethanolic extracts, using standard methods. The free radical scavenging potential of these extracts was assessed through 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), Total Antioxidant Capacity (TAC) and hydroxyl free radical assays.

Comparative analysis of the data revealed that the IC50 values for the three extracts were as follows: The aqueous extract had DPPH: 0.11 mg/mL, FRAP: 0.62 mg/mL and TAC: 0.40 mg/mL; the carbonated drink extract had DPPH: 10.17 mg/mL, FRAP: 0.68 mg/mL and TAC: 0.41 mg/mL; while the ethanolic extract had DPPH: 0.42 mg/mL, FRAP: 0.69 mg/mL and TAC: 0.35 mg/mL.

These values were lower than those of the standard (ascorbic acid), which had DPPH: 0.71 mg/mL, FRAP: 0.83 mg/mL and TAC: 0.65 mg/mL, except for the DPPH assay of the aqueous and carbonated drink extracts, where their IC50 values were higher than that of the standard.

The low IC_{50} values indicate that the plant extracts possess better antioxidant activity than the standard, suggesting that these extracts have significant antioxidant potential. Among the three extracts tested, the ethanolic extract demonstrated superior antioxidant potential compared to both the aqueous and carbonated drink extracts.

Keywords: *Andrographis paniculata*, 2,2-diphenyl-1-picrylhydrazyl, Ethylenediamine tetra acetic acid, Flavonoids, Andrographolide

***Correspondence:** Onyeneke Eusebius Chukwu, Department of Biochemistry, University of Benin, Benin city, Nigeria, E-mail: oluwaropoboluwade2019@gmail.com

INTRODUCTION

Andrographis paniculata, commonly known as chirayetah in the Indian subcontinent and Kalmegh in urdu and hindi, is an annual herb widely used in traditional medicine systems such as unani and ayurveda (Akbar S, 2011). This herbaceous plant belongs to the family Acanthaceae and is native to India, Taiwan and mainland China. It can also be found in tropical and subtropical regions of Asia, Southeast Asia and various other countries, including Cambodia, the Caribbean Islands, Indonesia, Laos, Malaysia, Myanmar, Sri Lanka, Thailand and Vietnam (Hossain MS, *et al*., 2014). In Southern and Southeastern Asia, it is extensively cultivated for the treatment of infections and various diseases (Anil K, *et al*., 2012).

Andrographis paniculata is recognized globally as an important medicinal plant with numerous local names. In English, it is referred to as the king of bitters. Other names include Mahatikta in Sanskrit, Kiryato in Gujarati, Kalmegh in India, Chuan-xin-lian in China, Fah thalai in Thailand, Hempedubumi in Malaysia, Senshinren in Japan, Sambiloto in Indonesia and Green chiretta in Scandinavian countries (Okhuarobo A, *et al*., 2014; Sivananthan M and Elamaran M, 2013). In Benin, it is locally known as oyinbo bitter leave, while the yoruba-speaking natives of Nigeria refer to it as Meje-Meje (seven-seven leaves).

Andrographis paniculata is a branched herbaceous plant that typically reaches a height of 60-70 cm. It features glabrous leaves measuring 8 cm long and 2.5 cm wide, with flowers that have minute white petals adorned with purplish spots (Bhaisare S, *et al*., 2023; Ameh SJ, *et al*., 2010). The plant grows erect to a height of 30-110 cm in moist shady locations. Its stem is dark green and ranges from 2-6 mm in diameter. The seeds are small, yellowish-brown and possess a bitter taste.

The capsules are erect and elliptical, measuring 1-2 cm long and 2-5 mm wide; they are compressed with longitudinal grooves on their broad faces and sharp at both ends. The capsules are sparsely glandular-hairy, while the seeds are sub quadrate and exceedingly tiny (Bhaisare S, *et al*., 2023).

However, the biological activities of *Andrographis paniculata* can be attributed to the presence of approximately 142 secondary metabolites, including entalabdane diterpenoids, flavonoids, quinic acid derivatives, xanthones, rare noriridoids, steroids and other compounds found in the plant's tissues. Most of these metabolites are extracted from the aerial parts, leaves and whole plant, while some are obtained from the roots (Jarukamjorn K and Nemoto N, 2008; Adiguna SB, *et al*., 2021).

Investigations into its chemical composition have shown that *Andrographis paniculata* is a rich source of diterpenoids and 2-oxygenated flavonoids such as andrographolide, neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, 14-deoxyandrographolide, isoandrographolide, 14-deoxy-andrographolide-19-β-D-glucoside, homoandrographolide, andrographan, andrographosterin and stigmasterol.

The aerial part of the plant contains andrographolide, neo-andrographolide and 14-deoxygenated andrographolide, while the roots primarily contain flavonoids.

Notably, andrographolide is the major bioactive constituent of *Andrographis paniculata* in terms of both bioactive properties and abundance (Chao WW and Lin BF, 2010). The leaves contain the highest concentration of andrographolide compounds at 2.39%, whereas the seeds have a relatively low content of this compound (Jayakumar T, *et al*., 2013).

Furthermore, Andrographolide exhibits free radical scavenging

activity and anti-inflammatory effects by inhibiting lipopolysaccharide-induced Nitric Oxide (NO) production and Inducible Nitric Oxide Synthase (INOS) expression (Brand WW, *et al*.,1995). It also suppresses Interleukin-2 (IL-2) production and T-cell proliferation (Tundis R, *et al*., 2023).

Additionally, Andrographolide is responsible for the extreme bitter taste of *Andrographis paniculata*.

This research aims to investigate the antioxidant potential of *Andrographis paniculata*.

MATERIALS AND METHODS

Chemicals and reagents

The following chemicals and reagents were used in this study, all of which are of standard analytical grade: absolute ethanol, acetic acid, ascorbic acid, distilled water, DPPH, Ethylenediamine Tetraacetic acid (EDTA), Ferric Tripyridyltriazine (Fe^{3+}-TPTZ), hydrogen peroxide, molybdate ions, sodium acetate, sodium bicarbonate, sodium carbonate, sodium dihydrogen phosphate, sodium hydrogen phosphate, sulfuric acid, sodium phosphate, potassium dihydrogen phosphate, potassium phosphate and methanol (Rahmi EP, *et al*., 2022)

Sample collection and authentication

The *Andrographis paniculata* plant was collected from the University of Benin environment 6.3998°N, 5.6099°E, Benin City, Edo State, in February 2024. The plant was identified and authenticated from the herbarium unit of the plant biology and Biotechnology Department, Faculty of Life Sciences, University of Benin, Nigeria. A voucher specimen with the number University Botanic Herbarium (UBH)-A599 was deposited in the herbarium unit for reference purposes.

Preparation and extraction of plant extracts

Fresh leaves and stems of *Andrographis paniculata* were selected, washed with distilled water and air-dried at room temperature. The dried plant materials were then pulverized into a homogeneous powder at the Pharmacognosy laboratory, Faculty of Pharmacy, University of Benin. 1 kg each of the pulverized leaves and stems was soaked in 1.5 L of aqueous solution, carbonated drink (7UP) and ethanol solvents, respectively. The resulting mixture was stirred for a few minutes using a clean wooden stirrer. The container containing the extract was then covered and allowed to extract for 72 hours with periodic agitation at room temperature. This procedure was repeated twice, after which the solvents were decanted and pooled. The resulting solution was filtered into clean containers using muslin cloth. The filtrate was concentrated to dryness using a rotary evaporator at a maximum temperature of 68°C. The resultant extracts were stored for further analyses at -4°C.

Statistical analysis

All results were subjected to descriptive statistics and are expressed as mean ± Standard Error of the Mean (SEM). One-Way Analysis of Variance (ANOVA) was used to analyze significant differences between the means of different groups. This was followed by Tukey's tests for pairwise comparisons and separation of means, with $p<0.05$ considered statistically significant.

RESULTS AND DISCUSSION

Antioxidants are molecules and substances capable of counteracting and slowing down oxidative reactions caused by free radicals (Prieto P, *et al*., 1999). They inhibit the chain reaction of oxidation by acting as hydrogen donors or acceptors from free radicals, thereby generating stable compounds (Guan HDU, 2018). Studies conducted in various parts of the world clearly support that bioactive substances extracted from *Andrographis paniculata* leaves using organic solvents contain phytochemicals with potential antioxidant activity.

Andrographolide, an important bioactive compound in *Andrographis paniculata*, contributes to the plant's antioxidant properties by directly neutralizing generated free radicals or indirectly protecting the mitochondria by inhibiting pro-oxidant enzymes and/or activating antioxidant enzymes. In this study, the antioxidant activity of *Andrographis paniculata* extracts (aqueous, carbonated drink and ethanolic) was assessed using standard methods, along with the estimation of the IC_{50} for each plant extract and the standard (ascorbic acid).

Table 1 presents the results of the DPPH *in vitro* antioxidant scavenging activity of the aqueous, carbonated drink and ethanolic extracts of *Andrographis paniculata* compared to ascorbic acid.

The results indicate that the carbonated drink extract exhibits the highest percentage inhibition among the three extracts when compared with the standard ascorbic acid, which shows a concentration-dependent percentage inhibition. However, the low IC_{50} value (0.11 mg/mL) of the aqueous extract suggests that it has the highest antioxidant capacity compared to the carbonated drink extract and ethanolic extract of *Andrographis paniculata*, which have IC_{50} values of 10.17 mg/mL and 0.42 mg/mL, respectively. This finding aligns with the results reported by (Hariharan T and Vasan P, 2023), who investigated the *in vitro* antioxidant activity of aqueous and alcoholic extracts of *Andrographis paniculata* whole plant powder using the DPPH free radical scavenging assay. In their study, the estimated IC_{50} values for ethanolic and aqueous extracts of *Andrographis paniculata* were 0.682 mg/mL and 0.292 mg/mL, respectively.

Table 2 presents the results of the FRAP percentage inhibition of the aqueous, carbonated drink and ethanolic extracts of *Andrographis paniculata* compared to ascorbic acid (Benzie IF and Strain JJ, 1996). It can be observed that the aqueous extract exhibits the highest percentage inhibition among the three extracts, followed by the carbonated drink extract, while the ethanolic extract shows the least percentage inhibition. Moreover, the low IC_{50} value 0.62 mg/mL of the aqueous extract indicates that it has the highest antioxidant capacity compared to the carbonated drink and ethanolic extracts, which have IC_{50} values of 0.68 mg/mL and 0.69 mg/mL, respectively. This result contrasts with the findings of (Akilandeswari G, *et al*., 2020), who reported that the ethanolic extract of *Andrographis paniculata* had a lower IC_{50} value, suggesting greater FRAP antioxidant activity compared to the aqueous extract. The discrepancies may be attributed to differences in specimens used and other environmental factors affecting plant growth and development.

Table 3 shows the results of the TAC percentage inhibition activity for the aqueous, carbonated drink and ethanolic extracts of *Andrographis paniculata* alongside ascorbic acid. The table demonstrates a concentration-dependent increase in percentage inhibition for all extracts of *Andrographis paniculata* and ascorbic acid, with ascorbic acid exhibiting the highest inhibition. There is no significant difference $p>0.05$ in percentage inhibition between the aqueous extract and ethanolic extract at concentrations of 0.8 mg/mL and 1.0 mg/mL. However, the estimated low IC_{50} value 0.35 mg/mL for the ethanolic extract indicates that it has the highest antioxidant potential compared to the carbonated drink, aqueous extract and ascorbic acid, which have IC_{50} values of 0.41 mg/mL, 0.40 mg/mL and 0.65 mg/mL, respectively.

Table 4 presents the results of hydroxyl radical scavenging activity for the aqueous, carbonated drink and ethanolic extracts of *Andrographis paniculata*. The results indicate a concentration-dependent increase in hydroxyl radical scavenging activity among the extracts. The aqueous extract demonstrates the highest scavenging activity, while the ethanolic extract shows the lowest scavenging activity at all concentrations tested. However, there is no significant difference $p>0.05$ in scavenging activity between the aqueous and carbonated drink extracts at concentrations of 0.2 mg/L and 0.6 mg/mL. Additionally, there is no significant difference among the estimated IC_{50} values for the three extracts.

Table 1: DPPH scavenging activity of *Andrographis paniculata* extracts and ascorbic acid

Concentration (mg/mL)	Aqueous extract	Carbonated drink extract	Ethanolic extract	Ascorbic acid
0.2	18.87 ± 2.90^{a}	21.41 ± 3.69^{b}	10.54 ± 3.74^{c}	95.67 ± 0.26^{d,e}
0.4	17.23 ± 3.82^{a}	20.94 ± 1.93^{b}	12.43 ± 6.25^{c}	96.22 ± 0.27^{e}
0.6	16.03 ± 5.96^{a}	23.77 ± 6.23^{b}	25.59 ± 7.74^{c}	94.92 ± 0.45^{d}
0.8	18.68 ± 2.77^{a}	17.70 ± 4.91^{b}	20.39 ± 2.46^{c}	95.75 ± 0.21^{d,e}
1	15.01 ± 4.25^{a}	20.32 ± 2.08^{b}	19.38 ± 12.80^{c}	97.37 ± 0.42^{f}
IC_{50}	0.11	10.17	0.42	0.71
Note: Data represent the mean ± standard deviation; Values with different superscripts in the same row are significantly different p<0.05				

Table 2: Fluorescence Recovery After Photobleaching (FRAP) scavenging activity of *Andrographis paniculata* extracts and ascorbic acid

Concentration (mg/mL)	Aqueous extract	Carbonated drink extract	Ethanolic extract	Ascorbic acid
0.2	31.65 ± 0.25^{a}	25.79 ± 0.40^{b}	22.53 ± 0.88^{c}	38.95 ± 0.82^{d}
0.4	25.01 ± 0.70^{a}	23.75 ± 2.00^{b}	21.05 ± 1.53^{c}	39.34 ± 0.12^{d}
0.6	23.61 ± 1.36^{a}	22.79 ± 1.00^{b}	22.09 ± 0.91^{c}	37.87 ± 1.26^{d}
0.8	24.50 ± 2.35^{a}	23.90 ± 1.00^{b}	20.06 ± 0.43^{c}	38.80 ± 0.44^{d}
1	22.10 ± 0.52^{a}	23.19 ± 1.00^{b}	21.13 ± 0.97^{c}	39.51 ± 0.77^{d}
IC_{50}	0.62	0.68	0.69	0.83
Note: Data represent the mean ± standard deviation; Values with different superscripts in the same row are significantly different p>0.05				

Table 3: Total Antioxidant Capacity (TAC) of *Andrographis paniculata* extracts and ascorbic acid

Concentration (mg/mL)	Aqueous extract	Carbonated drink extract	Ethanolic extract	Ascorbic acid
0.2	61.28 ± 10.17^{a}	35.97 ± 3.59^{d}	77.39 ± 3.78^{f}	91.92 ± 0.11^{h}
0.4	75.52 ± 0.51^{b}	40.09 ± 4.66^{d}	85.43 ± 2.23^{g}	91.89 ± 0.16^{h}
0.6	85.86 ± 0.60^{b,c}	71.74 ± 9.32^{e}	89.25 ± 1.55^{g}	91.82 ± 0.17^{h}
0.8	89.43 ± 2.02^{c}	76.66 ± 9.81^{e}	89.17 ± 1.26^{g}	91.90 ± 0.13^{h}
1	90.79 ± 1.17^{c}	74.76 ± 5.90^{e}	89.81 ± 1.35^{g}	92.03 ± 0.25^{h}
IC_{50}	0.4	0.41	0.35	0.65
Note: Data represent the mean ± standard deviation; Values with different superscripts in the same row are significantly different p>0.05				

Table 4: Hydroxyl radical scavenging activity of *Andrographis paniculata* extracts and ascorbic acid

Concentration mg/mL	Aqueous extract	Carbonated drink extract	Ethanolic extract
0.2	44.07	44.07	38.98
0.4	49.15	47.46	45.76
0.6	49.15	49.15	32.2
IC_{50}	2.95	2.95	2.95

CONCLUSION

The study has shown that *Andrographis paniculata* extracts (aqueous, carbonated drink and ethanolic) possess high antioxidant capacity, with the aqueous extract exhibiting the highest potency, followed by the ethanolic extract, while the carbonated drink extract has the least antioxidant capacity.

REFERENCES

1. Akbar S. *Andrographis paniculata*: A review of pharmacological activities and clinical effects. Altern Med Rev. 2011; 16(1): 66-77.
2. Hossain MS, Urbi Z, Sule A, Rahman KH. *Andrographis paniculata* (Burm. f.) Wall. ex Nees: A review of ethnobotany, phytochemistry, and pharmacology. Sci World J. 2014; (1): 274905.
3. Anil K, Jyotsna D, Anup S, Rishikant T. A review on king of bitter (Kalmegh). 2012.
4. Okhuarobo A, Falodun JE, Erharuyi O, Imieje V, Falodun A, Langer P. Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: A review of its phytochemistry and pharmacology. Asian Pac J Trop Dis. 2014; 4(3): 213-222.
5. Sivananthan M, Elamaran M. Medicinal and pharmacological properties of *Andrographis paniculata*. Int J Biol Macromol. 2013; 3: 1-2.
6. Bhaisare S, Pathak S, Ajankar VV. Physiological activities of the king of bitters (*Andrographis paniculata*): A review. Cureus. 2023; 15(8).
7. Ameh SJ, Obodozie OO, Inyang US, Abubakar MS, Garba M. A Normative study of Nigerian grown maha-tita (king of bitters)-*Andrographis paniculata* nees. Int J Drug Dev Res. 2010; 2(2): 291-299.
8. Jarukamjorn K, Nemoto N. Pharmacological aspects of *Andrographis paniculata* on health and its major diterpenoid constituent andrographolide. J Health Sci. 2008; 54(4): 370-381.
9. Adiguna SB, Panggabean JA, Atikana A, Untari F, Izzati F, Bayu A, *et al*. Anti-viral activities of andrographolide and its derivatives: Mechanism of action and delivery system. Pharmaceuticals. 2021; 14(11): 1102.
10. Chao WW, Lin BF. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). Chin Med. 2010; 5: 1-5.
11. Jayakumar T, Hsieh CY, Lee JJ, Sheu JR. Experimental and clinical pharmacology of *Andrographis Paniculata* and its major bioactive phytoconstituent andrographolide. Evid Based Complement Alternat Med. 2013; (1): 846740.
12. Brand WW, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol. 1995; 28(1): 25-30.
13. Tundis R, Patra JK, Bonesi M, Das S, Nath R, Das Talukdar A, *et al*. Anti-cancer agent: The labdane diterpenoid-andrographolide. Plants. 2023; 12(10): 1969.
14. Rahmi EP, Kumolosasi E, Jalil J, Buang F, Jamal JA. Extracts of *Andrographis Paniculata* (burm. f.) nees leaves exert anti-gout effects by lowering uric acid levels and reducing monosodium urate crystal-induced inflammation. Front Pharmacol. 2022; 12: 787125.
15. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 1999; 269(2): 337-341.
16. Guan HDU. Natural Small Molecule Drugs from Plants. Berlin/ Heidelberg, Germany: Springer. 2018.
17. Hariharan T, Vasan P. *In vitro* antioxidant activity of aqueous and alcoholic extracts of *Andrographis paniculata* whole plant powder by DPPH free radical scavenging assay. J Pharm Innov. 2023; 12(5): 3518-3521.
18. Benzie IF, Strain JJ. The Ferric Reducing Ability of Plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996; 239(1): 70-76.
19. Akilandeswari G, Bupesh G, Vijaya AA, Saradhadevi KM, Mayur MP. *In vitro* efficacy of antioxidant activity in ethanolic and aqueous leaf extracts of *Andrographis paniculata* nees and Rhinacanthus nasutus kurz. Int J Pharm Sci. 2020; 11(4): 6301-6306.