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In vitro antioxidant activity of aqueous and alcoholic extracts of *Andrographis paniculata* whole plant powder by DPPH free radical scavenging assay

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Abstract
The aim of the present investigation was to evaluate the antioxidant activities of whole plant extracts of *Andrographis paniculata* (APWP) by *in vitro* model DPPH free radical scavenging assay. Gallic acid, at a concentration of 0.5, 1.5, 2.0 and 2.5 µg/ml was used as reference inhibitor in DPPH assay which showed 6.55, 23.24, 44.32 and 78.72 percent inhibition of DPPH free radicals, respectively with IC_{50} value of 2.65 µg/ml (2.31- to 3.08 µg/ml). Both ethanolic and aqueous extracts of APWP at a concentration of 10, 25, 50, 100 and 200 µg/ml showed 0, 0, 2.38, 15.10 and 38.75 percent inhibition and 9.39, 13.80, 15.57, 24.96 and 39.07 percent inhibition of DPPH free radicals, respectively. The IC_{50} value of the ethanolic and aqueous extract samples in DPPH radical scavenging assay was 292.96 µg/ml (224.74 - 448.95 µg/ml) and 681.66 µg/ml (320.35 - 3518.99 µg/ml) respectively. So, the *in vitro* studies clearly showed that the whole plant extracts of *A. paniculata* has a significant antioxidant activity and the aqueous extract was found to be more effective in the hydroxyl radical scavenging activity with better IC_{50} value than the ethanolic extract of APWP. It could therefore be concluded that the free radical scavenging activity of the whole plant extract of *Andrographis paniculata* might be responsible for the therapeutic properties

Keywords: *Andrographis paniculata*, antioxidant activity, DPPH, free radical scavenging assay

Introduction
Free radicals are generally very reactive molecules possessing an unpaired electron which are produced continuously in cells either as by-products of metabolism or by leakage from mitochondrial respiration (Slater, 1984) [1]. The free radicals produced *in-vivo* include the active oxygen species such as super-oxide radical O_2^-, hydrogen peroxide (H_2O_2) and hypochlorous acid (HOCl). Oxygen free radicals have been shown to be responsible for many pathological conditions (De Zwart *et al*., 1999) [2]. Free radicals and Reactive Oxygen Species (ROS) cause DNA damage, lipid peroxidation and protein damage. They are known to be involved in the pathogenesis of a wide variety of clinical disorders such as cancer, cardiovascular diseases, inflammatory diseases, asthma and aging (Vani *et al*., 1997 [3] and Jadhav and Bhutani, 2002) [4]. Free radicals like the hydroxyl radical, hydrogen peroxide, super-oxide anion etc. mediate components of the inflammatory response, with production of migratory factors, cyclic nucleotides and eicosanoids. Super-oxide radicals amplify the inflammation process, increasing vascular permeability, adhesion of polymorphonuclear leucocytes to the endothelium and stimulation of platelet aggregation (Aragon *et al*., 1998) [5].
Plants and their products have abundant phytochemicals and have been proved to be good sources of potential antioxidants (Harman, 1992) [6]. Today consumers prefer natural antioxidants for use as nutraceuticals, biopharmaceuticals and as food additives (Rohman *et al.*, 2010) [7]. As these natural obtained antioxidants have fewer or no side effects, they are preferred over synthetically derived antioxidants which have genotoxic effects. Therefore, investigations of biological activity and chemical composition of medicinal plants as a potential source of natural antioxidants are numerous.
Andrographis paniculata (Burm. f.) Nees, a small annual herb, member of the family Acanthaceae, is found in Sri Lanka, Pakistan, Java, Malyasia, Indonesia and throughout India. In India, it is cultivated in Uttar Pradesh, Himachal Pradesh, Assam, Madhya Pradesh, Tamilnadu, Karnataka and Kerala. In Tamil Nadu, it is cultivated in Thanjavur, Salem, Erode, Vilupuram, Tiruchengode and Palayamkottai (Elumalai *et al.*, 2016) [8]. It is commonly known as Nila Vembu or Siriyanangai (Tamil), Kalmegh (Hindi) or King of bitters (English).

A. paniculata has been prominently used in at least 26 Ayurvedic formulations as confirmed from Indian Pharmacopoeia; It has been widely used in Chinese medicine as an anti-inflammatory and antipyretic drug for the treatment of cold, fever and laryngitis (Deng 1982) [9]. The plant is also one of the components of Nilavembu Kudineer Chooranam, a poly herbal Siddha preparation containing equal proportion of nine plants which is successful in the prevention and treatment of chikungunya, dengue viral fever and COVID-19 in human and hence approved for use by Government of India (Kavinilavan *et al.*, 2017) [10]. The plant has been reported to have several secondary metabolites having anti-oxidant properties with wide range of therapeutic applications.

Hence, the present study was formulated to study the *in-vitro* antioxidant activity of alcoholic and aqueous extracts of *A. paniculata* whole plant powder by *in vitro* method of DPPH free radical scavenging assays.

Materials and Methods

Collection of whole plants of *Andrographis paniculata*

Andrographis paniculata whole plants of around 120 days old were randomly collected as per the procedures of Jain, (2016) (11) from Herbal Garden of Department of Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal and District Forest Office (DFO), Mohanur Road, Namakkal (Situated at an average elevation of 218 metres above mean sea level with latitude of 11.23^0 North and longitude of 78.17^0 East), Tamil Nadu, India.

Authentication of the plant

The collected whole plants of *A. paniculata* were authenticated for its family and species by Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India.

Preparation of *A. paniculata* whole plant powder (APWP)

The collected whole plants and roots were washed under running tap water, spread on wetting papers and dried under shade for four weeks with frequent tilting. The whole plants were chaffed and further shade dried for another one week followed by mechanical grinding. Then the whole plants were pulverised and sieved to get a fine powder (Rajat Chakraborty and Tilottama Dey, 2016) [12]. The whole plant powder was stored in air tight containers for extract preparations.

Preparation of extracts of *A. paniculata* whole plant powder

Aqueous and alcoholic extracts of six samples of *A. paniculata* whole plant powder was prepared by adding 20 grams of dry powder to 200 ml of distilled water and 70 percent ethanol, respectively that was kept in a rotary shaker for 48hrs, filtered through Whatman No.1 filter paper and then incubated at 50 °C for 48hrs to evaporate the solvents. The dried extract was collected (Malahubban *et al*., 2013 [13] and Amin Mir *et al*., 2016) [14] and stored in airtight container for further investigations.

DPPH free radical scavenging assay

Principle

DPPH [1, 1-diphenyl-2-picryl hydrazyl] is a stable free radical with purple colour. Anti-oxidant reduces DPPH to 1, 1-diphenyl-2-picryl hydrazine, a colourless compound which is measured at an absorbance of 510 nm.

Materials required

- DPPH (1, 1-diphenyl-2-picryl hydrazyl, D9132, Sigma, USA, (Store at -20°C).
- Positive control: Gallic acid [3, 4, 5-Trihydroxy benzoic acid] (G7384, Sigma, USA, store at RT)
- Microwell plate - 96well flat, clear plate (Cat. no. 980040, Tarsons)
- Methanol (HPLC grade, M0275, Ranbaxy, India, store at RT)

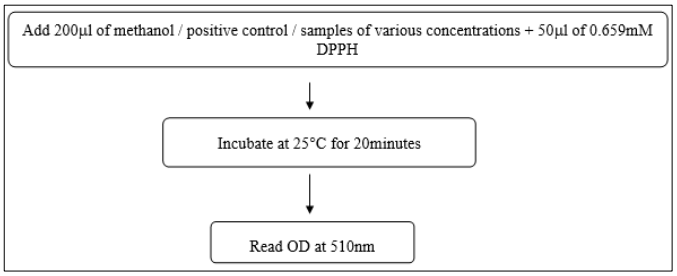
Preparation of working solutions

- DPPH (0.659mM): 2.6 mg is dissolved and made up to 10ml in HPLC grade methanol.
- Positive control (200µg/ml): 2 mg of Gallic acid is dissolved and made up to 10 ml HPLC grade methanol.

Procedure

DPPH assay is carried out as per the method of Vani *et al.*, 1997 [3]. In brief, a 250 µl total reaction volume contains 200µl of methanol / positive control /various concentration of test solution and 50 µl of 0.659 mM DPPH. The reaction mixture is incubated at 25 °C for 20 minutes, following which the absorbance is read at 510nm using micro-well plate reader (Molecular devices Versamax microplate reader).

Assay flow diagram



Calculation of results

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

Decrease in absorbance in the presence of test compounds at different concentrations was noted after 15 minutes. IC50 (i.e., the concentration of the test solution required to give a 50 percent decrease in absorbance, compared to that of blank solution) were calculated from percent inhibition. Gallic acid was used as a standard.

Results and Discussion

DPPH free radical scavenging assay

The results of *in-vitro* evaluation of anti-oxidant activities of aqueous and ethanolic extracts of APWP by DPPH free radical scavenging assay are presented in Table 1 and the 96-well plate used for this study depicted in Plate 1.

In this study, ethanolic and aqueous extracts of APWP were evaluated for its possible DPPH free radical scavenging activity. Gallic acid, at a concentration of 0.5, 1.5, 2.0 and 2.5 µg/ml was used as reference inhibitor which showed 6.55, 23.24, 44.32 and 78.72 percent inhibition of DPPH free radicals, respectively with IC_{50} value of 2.65 µg/ml with a range of 2.31- to 3.08 µg/ml.

Both ethanolic and aqueous extracts of APWP were tested for

its DPPH free radical scavenging activity at a concentration of 10, 25, 50, 100 and 200 µg/ml which showed 0, 0, 2.38, 15.10 and 38.75% inhibition and 9.39, 13.80, 15.57, 24.96 and 39.07% inhibition of DPPH free radicals for ethanolic and aqueous extracts, respectively.

The IC_{50} value of the ethanolic and aqueous extract sample in DPPH radical scavenging assay was found to be 681.66 µg/ml with a range of 320.35-3518.99 µg/ml and 292.96 µg/ml with a range of 224.74 - 448.95 µg/ml, respectively.

The antioxidant properties demonstrated by this study confirmed the previous findings of several researchers (Lin *et al*., 2009; Prakash *et al*., 2011; Sharma and Joshi, 2011; Adegboyega and Oyewole, 2013; Premanath and Nanjaiah, 2015; Low *et al*., 2015; Sivakumar and Rajeshkumar, 2015 and Sinha and Raghuwanshi, 2020) [15-22].

The findings of this study are in contrary with Adegboyega and Oyewole (2013) [18] who reported that the total phenolic content and DPPH free radical scavenging activity were higher in ethanolic extract when compared to aqueous extract. The results of this study corroborated with Low *et al*., (2015) [20] who used, the DPPH radical scavenging assay to assess the antioxidant capacity of the AP extracts. Sinha and Raghuwanshi (2020) [22] who confirmed the whole plant extract of *A. paniculata* as an important natural source of antioxidants and phytochemicals is in concord with the present study.

Aqueous extract of APWP showed better antioxidant activity that is primarily attributed to higher amount of polyphenolic phytochemicals present in aqueous extracts. Tannins which are water soluble, high molecular weight phenolic compounds are not only primary antioxidant donating hydrogen atom or electron, but also function as secondary antioxidants. The major pharmacological activity of *A. paniculata* resides in the diterpene compound (Andrographolide) which was shown to exhibit better antioxidant activity than ethanolic and aqueous extracts. Despite the presence of all these active principles the antioxidant activity was less than the standard antioxidant (Gallic acid) tested, which could be improved by adopting better extraction procedures.

Table 1: *In-vitro* antioxidant activity of ethanolic and aqueous extracts of APWP by DPPH free radical scavenging assay

Sample	Conc. (µg/ml)	%Inhibition	IC_{50} (µg/ml) (95% C.I.)
Gallic Acid (Reference Inhibition)	0.5	6.55	2.65 (2.31-3.08)
	1.5	23.24	
	2.5	44.32	
	5	78.72	
Ethanol extract	10	0	681.66 (320.35-3518.99)
	25	0	
	50	2.38	
	100	15.10	
	200	38.75	
Aqueous extract	10	9.39	292.96 (224.74-448.95)
	25	13.80	
	50	15.57	
	100	24.96	
	200	39.07	

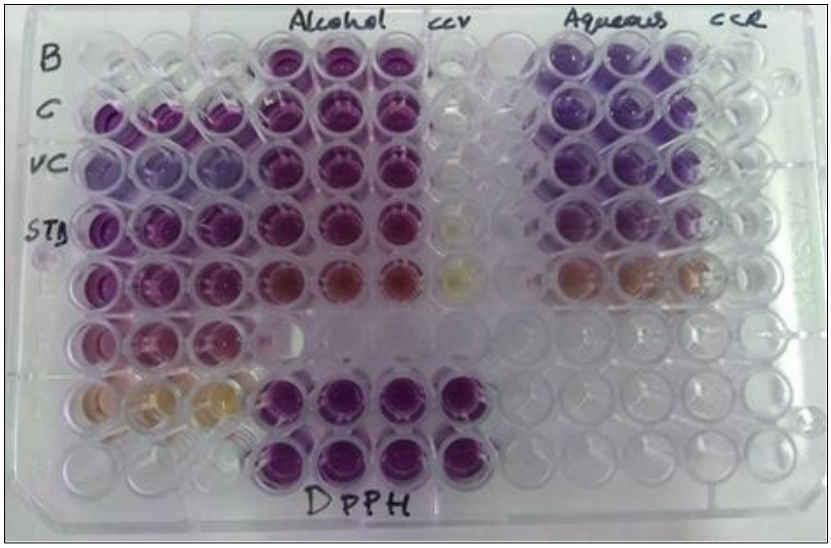


Plate 1: DPPH assay - 96 well plate

Conclusion

The results of the present study cleared showed that both ethanolic and aqueous extracts of APWP have potent anti-oxidant properties, which can be used as natural antioxidants for treatment of wide variety of disease conditions ranging from pyrexia to COVID-19 (Lim *et al.*, 2021) [23] not only in human and but also in animals.

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References

1. Slater TF. Free radical mechanisms in tissue injury. Biochem. J. 1984;222:1-15.
2. De Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and in humans. Free Rad. Biol. Med. 1984;26(1-2):202-226.
3. Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the Ayurvedic for- mulation-Triphala and its constituents. Int. J Pharmac. 1997;35(5):313-317.
4. Jadhav HR, Bhutani KK. Antioxidant properties of Indian Medicinal plants. Phytother. Res. 2002;16:771-773.
5. Aragon SM, Basabe B, Benedi JM, Villar AM. Antioxidant action of Vaccinium myrtillus L. Phytother

Res. 1998;12:S104-S106.
6. Harman D. Role of free radicals in aging and disease. Ann. New York Acad. Sci. 1992;673:598-620.
7. Rohman A, Riyanto S, Yuniarti N, Saputra WR, Utami R. Antioxidant activity, total phenolic, and total flavonoid of extract and fractions of red fruit (*Pardanus conoideus* Lam). Int. Food Res. 2010;17:97-106.
8. Elumalai S, Banupriya R, Sangeetha T, Madhumathi S. Review on psychopharmacological activity of Andrographis paniculata. Int. J Pharm. Bio. Sci. 2016;7(4):183-200.
9. Deng WL, Nie RJ, Liu JY. Comparison of pharmacological effect of four and rographolides. Chin. Pharm. Bull. 1982;17:195-198.
10. Kavinilavan R, Mekala P, Raja MJ, Arthanari Eswaran M, Thirumalaisamy G. Exploration of immuno-modulatory effect of Nilavembu Kudineer Chooranam against Newcastle disease virus in backyard chicken. Journal of Pharmacognosy and Phytochemistry. 2017;6(6):749-751.
11. Jain SK. A manual of Ethnobotany. 2nd Rev. Ed., Scientific Publisher, Jodhpur, India; c2016.
12. Rajat Chakraborty, Tilottama Dey. Drying protocols for traditional medicinal herbs: A critical review. International Journal of Engineering Technology, Management and Applied Sciences. 2016;4(4):312-319.
13. Malahubban M, Alimon AR, Sazili AQ, Fakurazi S, Zakry FA. Phytochemical analysis of *Andrographis paniculata* and *Orthosiphon stamineus* leaf extracts for their antibacterial and antioxidant potential. Trop. Biomed. 2013;30(3):467-480.
14. Amin Mir M, Sawhney SS, Manmohan Singh Jassal. Antimicrobial activity of various extracts of *Taraxacum officinale*. J Microb. Biochem. Technol. 2016;8(3):210-215.
15. Lin FL, Wu SJ, Lee SC, Ng LT. Antioxidant, anti-oedema and analgesic activities of Andrographis paniculata extracts and their active constituent andrographolide. Phytother Res. 2009;23:958-964.
16. Prakash ELS, Kadar Ali SH, Divya N, Reeta Vijaya Rani K, Manavalan R. Evaluation of *In-vitro* antioxidant activity of leaf extract of *Andrographis paniculata*. Res J Pharm Biol Chem Sci. 2011;2(2):891-895.
17. Sharma M, Joshi S. Comparison of anti-oxidant activity of *Andrographis paniculata* and *Tinospora cordifolia* leaves. J Curr. Chem. Pharm. Sci. 2011;1(1):1-8.
18. Adegboyega AM, Oyewole BM. Phytochemical screening and antimicrobial activities of leaf extracts of *Andrographis paniculata.* International Journal of Science and Research. 2013;4(6):1-3.
19. Premanath, Nanjaiah. Antidiabetic and antioxidant potential of *Andrographis paniculata* Nees. leaf ethanol extract in streptozotocin induced diabetic rats. J Appl. Pharm. Sci. 2015;5(1):069-076.
20. Low M, Khoo CS, Munch G, Govindaraghavan S, Sucher NJ. An *in vitro* study of anti-inflammatory activity of standardised *Andrographis paniculata* extracts and pure andrographolide. BMC Complement Altern. Med. 2015;15:18.
21. Sivakumar V, Rajeshkumar S. Protective effect of *Andrographis paniculata* on hyperglycemic mediated oxidative damage in renal tissues of diabetic rats. J Phytopharm. 2015;4(6):287-294.
22. Sinha S, Raghuwanshi R. Evaluation of phytochemical, antioxidant and reducing activity in whole plant extract of *Andrographis paniculata* (Burm. f.) Wall. ex Nees. Biosc. Biotech. Res. Comm. 2020;13(4).
23. Lim Xin Yi, Janice Sue Wen Chan, Terence Yew Chin Tan, Bee Ping Teh, Mohd Ridzuan Mohd Abd Razak, Saharuddin Mohamad, et al. *Andrographis paniculata* (Burm. F.) Wall. Ex Nees, Andrographolide, and Andrographolide analogues as SARS-COV-2 Antivirals? A rapid review. Natural Product Communications. 2021;16(5):1-15.