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Phytochemical analysis of *Andrographis paniculata* whole plant powder

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Abstract
Andrographis paniculata whole plants were collected and authenticated for its family and species. Its moisture and dry matter content ranged from 57.90 to 59.02 and 40.98 to 42.10 per cent, receptively. *A. paniculata* whole plant powder was prepared from dried *A. paniculata* whole plants and the ethanolic and aqueous extracts were prepared from *A. paniculata* whole plant powder. The yield of ethanolic extract (10.92±0.11 per cent) was significantly ($P \leq 0.01$) higher than the yield of aqueous extract (8.13±0.11 per cent). The ethanolic and aqueous extracts of *A. paniculata* whole plant powder were screened for fourteen phytochemicals of which saponins, tannins, phlobatannins, hydrolysable tannins, phenols, alkaloids, terpenoids, flavonoids and glycosides were present in aqueous extract. Ethanolic extract also showed similar results with the exception of cardiac glycosides which were present and phlobatannins which were not detected.

Keywords: *Andrographis paniculata*, ethanolic and aqueous extracts, yield, phytochemicals

Introduction
Andrographis paniculata (Burm. f.) Nees, is a small annual herb, member of the family ACANTHACEAE, found in Sri Lanka, Pakistan, Java, Malaysia, 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) [1]. 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) [2]. 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 (Kavinilavan *et al.*, 2017) [3] and COVID-19 in human and hence approved for use by Government of India. The plant has been reported to have several secondary metabolites with wide range of therapeutic applications. An attempt was made to identify the bioactive components present in the aqueous and ethanolic extracts of *A. paniculata* whole plant powder.

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 procedure of Jain, (2016) [4] from Herbal Garden of Department of Pharmacology and Toxicology, Veterinary College and Research Institute, Namakkal and District Forest Camp 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)

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.

Dry matter content of *A. paniculata* whole plant
Random samples from the collected whole plants were cut and kept in hot air oven for drying at 50 ºC for 24 hours for the estimation of dry matter (Rajat Chakraborty and Tilottama Dey, 2016) [5].

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) [5]. The whole plant power 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 (APWP) were prepared by adding 20 gram of dry powder to 200 ml of distilled water and 70 percent ethanol, respectively, that were and 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 and the percentage yield was calculated (Malahubban *et al*., 2013 and Amin Mir *et al*., 2016) [6, 7]. The collected material was stored in airtight container for phytochemical analysis.

Phyto-chemical analysis
Qualitative phyto-chemical analysis of aqueous and alcoholic extracts of APWP powder were carried out by using commonly employed precipitation and coloration reaction as per the methodology of Harborne, (1998) [8], Priyanka Das and Alok Kumar Srivastav, (2014) [9] and Lalitha *et al*., (2015) [10] at Ethno Veterinary Herbal Research Centre for Poultry (EVHRCP), Veterinary College and Research Institute, Namakkal, Tamilnadu, India which revealed the presence or absence of fourteen phytochemical compounds *viz*. saponins, tannins, phlobatannins, hydrolysable tannins, phenols, alkaloids, terpenoids, flavonoids, glycosides, cardiac glycosides, amino acids, carbohydrates, volatile oils and vitamin C.

a. Detection of alkaloids
To 2.0mL of each extract, 2.0 ml of picric acid (Hager's reagent) was added. The appearance of orange or yellow color precipitate is suggestive of alkaloids.

b. Detection of cardiac glycosides
To 2.0 ml of each extract, 2.0 ml of dilute H_2SO_4 was added and heated at 50˚C for 2 min. Then 1.0 ml of 10 per cent NaOH was added and 5.0 ml each of Fehling's solution A and B were added. The appearance of brick red precipitate is indicative of glycosides.

c. Detection of glycosides
To 2.0 ml of each extract, an equal amount of glacial acetic acid was added. Then, one drop of 10 percent ferric chloride and 2.0 ml of concentrated H_2SO_4 were added. The appearance of three layers of colours like upper green, middle brown and lower violet is suggestive of cardiac glycosides.

d. Detection of phenols
Two ml of each extract were diluted with 2.0 ml of 10 per cent ferric chloride. The appearance of bluish color indicates the presence of phenols.

e. Detection of tannins
To 2.0 ml of each extract, 3 drops of 1 per cent ferric chloride was added. The appearance of blue green color is suggestive of tannins.

f. Detection of phlobatannins
To 2.0 ml of each extract, 1.0 ml of dilute HCl solution was added. The appearance of red precipitate is indicative of phlobatannins.

g. Detection of hydrolysable tannins
To 2.0 ml of each extract, 2.0 ml of ammonia solution was added. The appearance of emulsion indicates the presence of hydrolysable tannins.

h. Detection of flavonoids
To 2.0 ml of each extract, few drops of sodium hydroxide solution were added. The appearance of intense yellow colour, which became colourless on addition of dilute HCl is suggestive of flavonoids.

i. Detection of terpenoids
To 2.0 ml of each extract, an equal amount of chloroform was added followed by addition of 2.0 ml of concentrated H_2SO_4 along the sides of the test tube. The appearance of a brown color ring at the junction of two liquids is indicative of terpenoids.

j. Detection of saponins
Two ml of each extract were diluted with 10.0 ml of distilled water and mixed for 15 minutes. The appearance of layers of foam which remains for 10 minutes is suggestive of saponins.

k. Detection of amino acids and proteins
To 2.0 ml of each extract, 0.25 percent w/v Ninhydrin reagent was added and boiled for 2 minutes. The appearance of violet or blue color is indicative of amino acids and proteins.

l. Detection of carbohydrates
To 2.0 ml of each extract, 2.0 ml each of Fehling's A and B solution were added and heated at 50˚C for 1 minute. The appearance of red precipitate is suggestive of carbohydrates.

m. Detection of volatile oil
To 2.0 ml of each extract, 0.1 ml of NaOH and a small amount of dilute HCl were added. The appearance of white precipitate is indicative of volatile oils.

n. Detection of vitamin C
To 2.0 ml of each extract, 2.0 ml of sodium bicarbonate solution was added. The appearance of violet color is suggestive of vitamin C.

Results and Discussion

Authentication of the plant
The samples of used *Andrographis paniculata* whole plant for analyses were authenticated as '*Andrographis paniculata* (Burm. f.) Nees' (Family - *ACANTHACEAE*) by the Botanical Survey of India, Southern Regional Centre, Tamil Nadu Agricultural University Campus, Coimbatore, Tamil Nadu, India.

Dry matter content of *A. paniculata* whole plant
The per cent dry mater and moisture contents of *A. paniculata* whole plant collected from Veterinary college and Research Institute (VCRI), Namakkal and District Forest Camp Office

(DFO), Namakkal is presented in the Table 1.

Table 1: Moisture and dry matter content of fresh *A. paniculata* whole plant

Sl. No	Plant Collected	Moisture (%)	Dry Matter (%)
1	VCRI, Namakkal	59.02	40.98
2	DFO, Namakkal	57.90	42.10

Each value is a mean of six observations

The moisture and dry mater content of collected samples ranged from 57.90 to 59.02 and 40.98 to 42.10 per cent, respectively.

Whereas, the range of moisture content were 73.02-71.65, 68.00 and 31.40 per cent and dry matter content were 26.98-28.35, 32.00 and 68.60 per cent respectively, in leaves, stems and seeds, as reported by Moyo *et al*. (2011) [11], Abasiekong and Osabor (2017) [12] and Geetha and Rajeswari (2019) [13]. The lower moisture and higher dry matter content of APWP when compared to the reported values might be due to the mixture of leaves, stems and roots.

Yield of aqueous and alcoholic extracts

The per cent yield of aqueous and alcoholic extracts of APWP is presented in Table 2 and Figure 1. The mean per cent yield of aqueous and alcoholic extracts of APWP samples were 8.13 ±0.11 and 10.92±0.11 per cent, respectively. The mean per cent yield of alcoholic extract was significantly ($P< 0.01$) higher than aqueous extract which indicated that APWP consisted of contains higher amount of alcohol soluble bioactive compounds than that of water soluble compounds.

Table 2: Per cent yield of extracts of *A. paniculata* whole plant powder

Sl. No	Aqueous extract yield (%)	Alcoholic extract yield (%)
1	7.74	11.32
2	8.47	10.93
3	8.22	10.66
4	8.39	10.78
5	7.98	11.15
6	8.00	10.68
Mean±SE	8.13 ±0.11^{b}	10.92±0.11^{a}

Means with different superscripts differ significantly ** ($P< 0.01$)

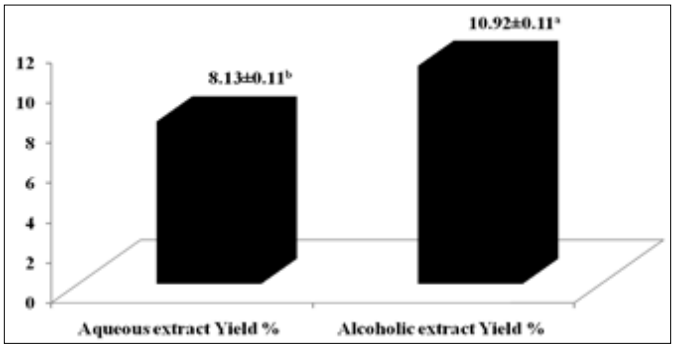

Fig 1: Percentage Yield (w/w) of aqueous and alcoholic extracts

The yield of aqueous and alcoholic extracts in the of present study were was lower than the reported values (Mohan *et al.*, 2013; Banji *et al.*, 2018 and Nagajothi *et al.*, 2018) [14, 15, 16] which were attributed to the fact that as the extracts of the present study were prepared from the dried whole plant powder where as in reported studies, the extracts were prepared only from dried leaves. But the yield of alcoholic extracts was comparable with the values from the aerial parts as reported by Mishra *et al.* (2009) [17].

Qualitative Phytochemical analysis

The results of qualitative phytochemical constituents of aqueous and alcoholic extracts of APWP are presented in the Table 3.

Qualitative analysis showed that saponins, tannins, phenols, alkaloids, terpenoids, flavonoids, hydrolysable tannins and glycosides were present both in aqueous and alcoholic extracts of APWP. Whereas, amino acids, carbohydrates, volatile oils and vitamin C were absent both in aqueous and alcoholic extracts of APWP.

Phytochemical screening of aqueous and ethanolic extracts of APWP showed positive for eight bioactive compounds of which phlobatannins and cardiac glycosides were detected only in aqueous and ethanolic extract, respectively. The probable reason might be due to the difference in extraction potential of the solvents (Polash *et al.*, 2017) [18]. The presence of alkaloids, phenols, tannins, hydrolysable tannins, flavonoids, terpenoids and saponins in both the extracts were earlier reported to be important for antiviral activity (Arbab *et al.*, 2017) [19].

Table 3: Qualitative phytochemical constituents of aqueous and alcoholic extracts of APWP

Sl. No	Phytochemicals	Aqueous extract	Alcoholic extract
1	Saponins	Present	Present
2	Tannins	Present	Present
3	Phenols	Present	Present
4	Alkaloids	Present	Present
5	Terpenoids	Present	Present
6	Flavonoids	Present	Present
7	Amino acids and Protein	Absent	Absent
8	Carbohydrates	Absent	Absent
9	Phlobatannins	Present	Absent
10	Volatile Oils	Absent	Absent
11	Hydrolysable tannins	Present	Present
12	Glycosides	Present	Present
13	Cardiac glycosides	Absent	Present
14	Vitamin C	Absent	Absent

Similar observations were reported by Malahubban *et al.* (2013)[6], Adedapo *et al.* (2014) [20], Umadevi and Kamalam, (2014) [21], Adegboyeg and Oyewole (2015) [22], Neha Sinha (2016) [23], Nagajothi *et al.* (2018) [14] and Bhargavi and Kalpana Kaloori (2018) [24] except for the absence of alkaloids as reported by Adedapo *et al.* (2014) [19] and absence of terpenoids as reported by Neha Sinha (2016) [19] and Bhargavi and Kalpana Kaloori (2018) [24] in aqueous extracts. Similarly, presence of phylobatannins in aqueous and presence of cardiac glycosides in alcoholic extracts of APWP were also earlier reported by Nagajothi *et al.* (2018) [14].

Conclusion

All these phytochemicals present in *A. paniculata* act synergistically and exhibit beneficial effects in treatment of wide variety of disease conditions ranging from pyrexia to cancer. Hence, *A. paniculata* is included as an ingredient in several polyherbal preparations for its heaptoprotective (Ram, 2001) [25], antiviral (Calabrese *et al.*, 2000)[26] and immunostimulant activity (Kavinilavan *et al.*, 2017)[3] for treatment of wide variety of disease conditions ranging from pyrexia to COVID-19 (Lim *et al.*, 2021) [27] not only in human and but also in animals.

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