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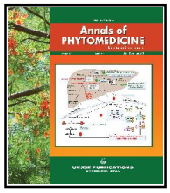

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Phytochemicals and antioxidant potential of *Andrographis paniculata* (Burm. f.) *Wall. ex Nees*

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Abstract

In the course of recent years, enthusiasm for therapeutic plants has developed tremendously. As a source of alternative medicines, plant derived drugs are of great potential and can be used to cure various health related ailments. In Ayurveda, plants of Acanthaceae family are of great medicinal importance. *Andrographis paniculata* Nees commonly known as Kalmegh (Dark cloud), belongs to family Acanthaceae, is a significant therapeutic, herb well known for its anti-inflammatory, antibacterial, antioxidant, anti-HIV properties and for cure of variety of other communicable diseases extending from malaria to dysentery. Ayurvedic and Homoeopathic medicine systems frequently use plant aerial parts and roots. Considering the above concerns, research was conducted to evaluate the chemical composition and antioxidant properties of *A. paniculata*. Aerial parts of the plant were collected and moisture content was estimated. The residual sample was then shade-dried, followed by oven drying, and again the moisture content was estimated. Various chemicals and phytonutrients like minerals, alkaloids, tannins, crude protein, crude fibre, flavonoids and total phenolics were present. After drying, the aerial parts were ground and solvent extracts of the aerial parts were prepared using different solvents with increasing polarities. Antioxidant activity was assessed from various prepared solvent extracts using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical method. Plant extract's antioxidant capacity was contrasted with that of regular ascorbic acid.

1. Introduction

Ever since the earth's creation, civilizations have employed medicinal plants to treat a variety of illnesses (Devi *et al*., 2020; Moond *et al*., 2023). In addition to this herbal approach, there is a widespread trend towards the use of herbal products as dietary supplements, primarily to improve quality of life and prevent disease (Goel *et al*., 2022; Moond *et al*., 2023). The secondary metabolites produced by medicinal plants, or bioactive constituents, are abundant in these plants (Moond *et al*., 2023). The non-nutritive secondary metabolites are known as phytochemicals that have defensive as well as disease preventive properties (Kumari *et al*., 2022; Moond *et al*., 2023). The main secondary metabolites include carbohydrates, flavonoids, alkaloids, terpenoids, steroids and tannins (Goel *et al*., 2022; Devi *et al*., 2023). In plants, the phytochemicals keep on modifying by each passing generation for better survival (Nehra *et al*., 2023). The need for new antibacterial agents has arisen from the development of bacterial resistance to existing antibiotics (Suman *et al*., 2022; Devi *et al*., 2023). Currently, natural sources of antioxidants are the main emphasis rather than potential synthetic antioxidants due to their adverse impacts on human health (Aggarwal *et al*., 2022).

Andrographis paniculata (Burm. f.) Wall. ex Nees belonging to family Acanthaceae is a herb, native to India and Sri Lanka. The cultivation of it is widespread in Southern and South-Eastern Asia (Goel *et al*., 2022). It is commonly known as Kalmegh (Kumar *et al*., 2012), Nilavembu (Rajalakshmi *et al*., 2012), Mahatikta in Sanskrit meaning "King of Bitters" (Alagesaboopathi, 2000). *A. paniculata* is one of the medicinal plants that is most frequently used in Unani and Ayurvedic medicine. The plant's aerial portion is most frequently used. The extract contains lactones, diterpenoids, diterpene glycosides, flavonoid glycosides and flavonoids (Hossain *et al*., 2014). Andrographolide, a diterpenoid lactone that is the primary bioactive component of *A. paniculata*, has demonstrated efficacy in the treatment of upper respiratory tract infections (Coon and Ernst, 2004). A study revealed that extracts of *A. paniculata* could increase the time of blood clotting in different solvents, thus decreasing the chances of consequent closing of blood vessels (retenosis) (Sani *et al*., 2016). The leaves and roots are also used as antivenom for cobra bite and other poisonous bites (Kirtikar and Basu, 1984). *A. paniculata* being a significant therapeutic plant, is well known for its anti-inflammatory (Hidalgo *et al*., 2005), anticarcinogenic (Chen and Liang, 1982), antidysentric (Akbar, 2011), antivenomic (Kirtikar and Basu, 1984), anti-HIV (Stephan and Comac, 2000), antiplamsodial (Dua *et al*., 2009), antifungal (Radha *et al*., 2011), and antidiabetic (Radhika *et al*., 2012) properties. The active ingredients andrographolide and neo-andrographolide present in plant prevents oxidative damage and inhibits binding of toxic metabolites to DNA (Kumar *et al*., 2012). Extraction plays an important role as it helps in the recovery of desired medicinally bioactive constituents from plants by using selective solvents and leaving out those non-desired with an aid of the solvents (Dhanani *et al*., 2017).

2. Materials and Methods

2.1 Plant material

Aerial samples of *A. paniculata* were obtained from the Department of Medicinal and Aromatic Plants of CCS Haryana Agricultural

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University, Hisar. The proposed study was conducted in Department of Chemistry, CCS HAU, Hisar. The plant material was brought and before processing, the material was kept at room temperature in the shade. The plant was identified and authenticated by Dr. Surender Yadav, Associate Professor, Department of Botany, Maharshi Dayanand University, Rohtak. Voucher Specimen No. VS/Phcog/215 was kept in the Department of Pharmacy for future reference.

$$\text{Moisture content (\%)} = \frac{\text{Powderedwt.}\left(\text{before drying}\right) - \text{Powdered wt.}\left(\text{after drying}\right)}{\text{Powderedwt.}\left(\text{before drying}\right)} \times 100$$

2.2.2 Estimation of ash content

Three replicates of 2 g of aerial *A. paniculata* powder sample were weighed and transferred to a crucible that had already been burned and weighed, and then placed in a muffle furnace for two hours. From the oven to the desiccator, the crucibles with the sample are transferred directly and allowed to cool and take their weight.

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

2.2.3 Estimation of crude fat content

2 g of aerial part of *A. paniculata* dried powder sample were taken from the extraction tube and retained in the Soxhlet extractor. A pre-weighed and dried round bottom flask (250 ml) is connected to the Soxhlet assembly. The petroleum ether is then poured into 1.5 syphons, or about 150-175 ml. The entire process is heated, and the extraction lasts for eight hours. Following extraction, the round-bottom flask's weight was reweighted with the extract after the kerosene ether was evaporated from the flask. The crude fat content (%) is calculated as follows:

$$\text{Crude fat content (\%)} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

2.2.4 Estimation of crude fibre content

3 g of moisture and fat-free powder samples from the aerial part of *A. paniculata* were taken. Thereafter, the percentage of crude fibre content was calculated by using modified method of Maynard (1970).

2.2.5 Estimation of crude protein content

Approximately 100 mg of the powder sample of the *A. paniculata* aerial fraction was taken and then the micro-Kjeldahl method (AOAC, 1990) was used to calculate the nitrogen content. Crude protein was determined by multiplying the nitrogen percentage by a 6.25 factor.

2.2.6 Total carbohydrates

The difference is used to calculate the total amount of carbohydrates as follows:

Total carbohydrates content (%) =100 - [Moisture (%) + Ash (%) + Crude fat (%) + Crude fibre (%) + Crude protein (%)]

2.3 Mineral analysis

The method developed by Jackson (1973) and Ruig (1986) was used to calculate the mineral content. Using an atomic absorption spectrometer, Fe, Zn, Mn, and Cu minerals in acid digested plant samples were measured. According to the AAS principle, when atoms of metallic elements (Fe, Zn, Mn, and Cu) are subjected to specific wavelength radiations, they become motivated and absorb energy despite ordinarily residing in the ground state. Each metallic element is illuminated by a different lamps. The amount of that element's atoms present is directly correlated with the amount of radiation that is absorbed. Atoms are not affected by the temperature or wavelength of the radiation they absorb.

2.2 Proximate analysis

2.2.1 Estimation of moisture content

In triplicate, about 2 g powdered sample of aerial part of *A. paniculata* were taken and method of AOAC (1995) was used to calculate the percentage of moisture content.

2.4 Chemical analysis

2.4.1 Determination of tannin content

In a 25 ml test tube, 200 mg of the powdered sample of the *A. paniculata* upper fraction was taken and 10 ml of methanol was added, and then Burns' Vanillin-HCl method (1971) was used to assess the tannin content in catechin equivalents.

2.4.2 Determination of alkaloid content

In a 250 ml beaker, about 2.5 g of the powdered samples of aerial part of *A. paniculata* were taken and method of Harborne (1973) was used for assessment of alkaloid content.

2.5 Extraction in aqueous, ethanol and ethyl acetate solvent of samples

In a Whatman No. 1 filter paper thimble, 10 g of the powder sample of the *A. paniculata* upper portion was taken and sited in a standard Soxhlet apparatus equipped with a 500 ml round-bottomed flask. Approximately 250 ml of solvent (distilled water, ethanol and ethyl acetate) was added to one and a half siphons. The corresponding solvent is used for boiling point extraction. In the column, the solvent vapor is transferred upward and flows into the extraction tube chamber filled with samples from the *A. paniculata* aerial section after condensing in the condenser. The syphon mechanism starts once the solvent has completely filled the chamber and contains some of the phytochemicals that have been dissolved in it. In the round bottom flask, this extract was emptied. In the case of ethanol and ethyl acetate as solvents using the siphon mechanism, the process was continued for 56 h after the completion of seven to eight cycles. On the other hand, distilled water as a solvent takes longer time for extraction through siphon mechanism so as to complete the 7-8 cycles, as its single cycle takes time more significantly. After first extraction was completed, the respective solvents were used for extraction, *i.e.,* about 2 h and 1 h of residue in thimble. The volume of each filtered solvent from three extraction steps was recorded. To determine the total number of phenolic compounds, the total number of flavonoids and the assessment of DPPH free radical scavenging activity wasperformed using aqueous, ethanol and ethyl acetate extracts.

2.6 Phytochemical parameters

2.6.1 Determination of total phenolics

The calibration or standard curve of gallic acid was created using a stock solution of gallic acid (60 g/ml) in methanol: water (50%, v/v) and subsequent dilutions to 10, 20, 30, 40, 50, and 60 g/ml of working solutions. 2.0 ml of sodium carbonate (20%, w/v) and 1.0 ml of 1mol/l Folin-Ciocalteu reagent were added to 1.0 ml of each concentration of standard solution. The mixture was then diluted with distilled water to a volume of 10.0 ml. The solution was incubated for 8 min, centrifuged at 6000 rpm for 10 min, and the supernatant solution was used to measure the absorbance at 730 nm using a UV-Vis double beam spectrophotometer (Model UV 1900, Shimadzu) in comparison to a blank that had been similarly prepared but had used distilled water in place of a standard solution of gallic acid.

2.6.2 Determination of total flavonoids

Each concentration of the standard solution was taken about 1ml to estimate the total number of flavonoids and added 4 ml of double distilled water, 0.3 ml of 5% $NaNO_2$ and after 5 min, 0.3 ml of 10% $AlCl_3$ was mixed. Immediately 2 ml of 1 M NaOH was added and used twice the volume made up to 10 ml with distilled water. As soon as 2 ml of 1 M NaOH had been added, 10 ml of distilled water had been doubled in volume. A UV-Vis model UV 1900 dual-beam spectrometer (Shimadzu) was used to measure the solution's absorbance at 510 nm after it had been thoroughly mixed. From the standard curve in the extracts, the amount of total flavonoids present was calculated using catechins, since the standard analysis of total flavonoids was performed using a colorimetric test against aluminum chloride, according to the explanation as given by Marinova *et al.* (2005) and expressed in milligrams of catechin equivalents per gram (mg CE/g).

2.7 Evaluation of antioxidant activity

DPPH free radical scavenging activity was assessed using the modified Hatano *et al.* (1988) technique. Each extract was lyophilized to produce the dry mass, and the dry mass was then dissolved in the appropriate solvent to create solutions of various concentrations. Then, a test tube containing 1 ml of each extract at the proper concentration received 2.0 ml of DPPH (0.1 mM in methanol). The mixture was thoroughly stirred for five minutes. Following incubation of the reaction mixture in the dark for 30 min at room temperature, the absorbance of the extract and control was measured at 517 nm using a UV-Vis double beam spectrophotometer (Model UV 1900, Shimadzu) against a blank containing the appropriate solvent. On a graph, the extract concentration (g/ml) and DPPH free radical scavenging activity (%) were plotted. The formula from the equation $ax^2 + bx + c = 0$ was used to calculate the Half-maximal Inhibitory concentration (IC_{50}). The IC_{50} was calculated by:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where,

$x = IC_{50}$ (µg/ml)

2.8 Statistical analysis

For statistical evaluation, the sample was collected in three copies. Using the statistical software SPSS (Statistical Package for Social Sciences) version 23, the data of proximate composition and phytochemicals were expressed as mean standard error (SE). The regression analysis of the IC_{50} values for antioxidant activity was evaluated using Microsoft Excel.

3. Results

In proximate composition, the aerial part of *A. paniculata* consist the moisture content (1.87 ± 0.02 %), crude fibre content (14.50 ± 0.02%), ash content (8.75 ± 0.14%), crude protein content (16.10 ± 0.23%), crude fat (8.91 ± 0.02%) and carbohydrates (49.87 ± 0.38%).

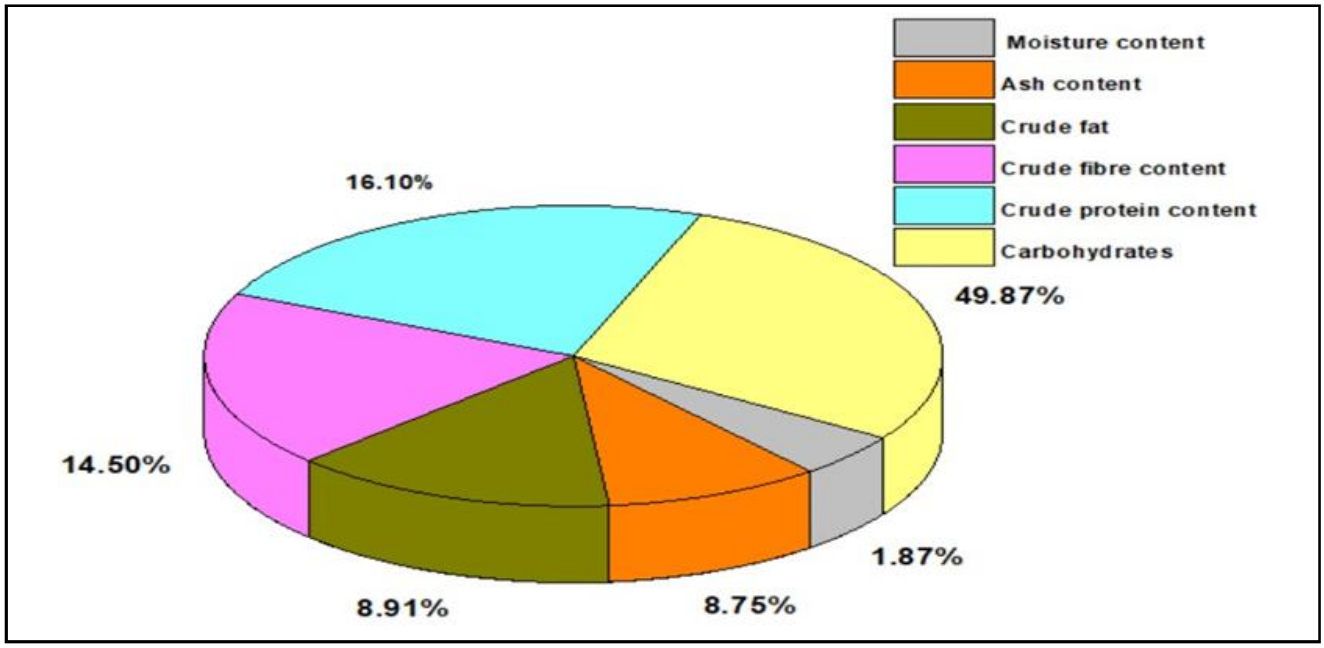

Figure 1: Proximate composition of aerial part of *A. paniculata*.

In mineral analysis, the mineral content in aerial part of *A. paniculata*, the minerals (Fe, Mn, Zn and Cu) content was estimated and data is presented in Table 1. The mineral content in aerial part of *A. paniculata* contains Fe (273.52 ± 8.32 ppm), Mn (11.82 ± 2.32 ppm), Zn (50.14 ± 4.37 ppm) and Cu (1.95 ± 0.45 ppm). However, till now no data is available for mineral content of aerial part and roots of *A. paniculata*. In chemical analysis, aerial part of *A. paniculata* contains the tannin content (3.69 ± 0.04 mg CE/g) and alkaloid content (1.82 ± 0.01%) as shown in Table 1.

Table 1: Mineral and chemical analysis of aerial part of *A. paniculata*

Mineral analysis (ppm)			
Fe	**Mn**	**Zn**	**Cu**
273.52 ± 8.32	11.82 ± 2.32	50.14 ± 4.37	1.95 ± 0.45
Chemical analysis			
Tannin content (mg CE/g)		**Alkaloid content (%)**	
3.69 ± 0.04		1.82 ± 0.01	

In phytochemical parameters, the content of total phenolics and total flavonoids were estimated in aerial part of *A. paniculata* (Figure 2). The total phenolics content in aerial part of *A. paniculata* among aqueous extract (2.03 ± 0.04 mg GAE/g), ethanol extract (0.67 ± 0.02 mg GAE/g) and ethyl acetate extract (0.39 ± 0.02mg GAE/g). The total flavonoids content in aerial part of *A. paniculata* among aqueous extract (1.78 ± 0.04 mg CE/g), ethanol extract (0.85 ± 0.05 mg CE/g) and ethyl acetate extract (0.69 ± 0.01mg CE/g).

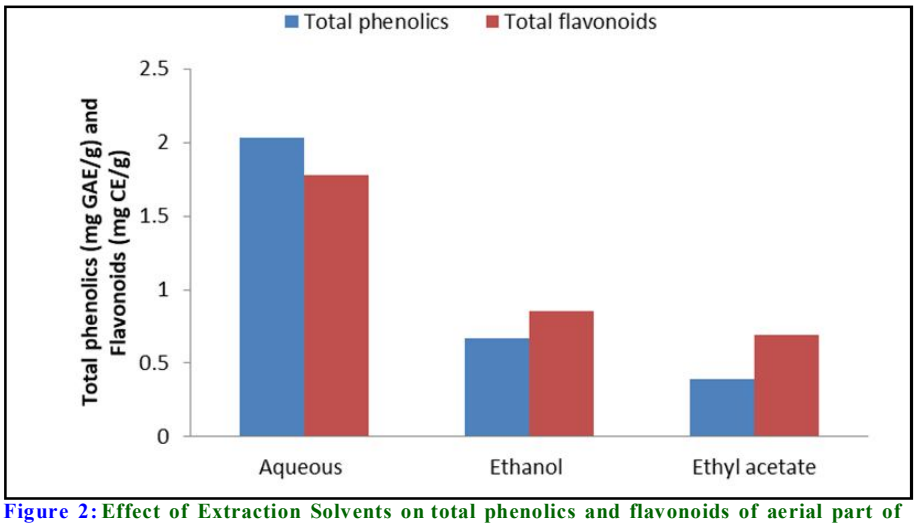

Figure 2: Effect of Extraction Solvents on total phenolics and flavonoids of aerial part of *A. paniculata*.

The percentage of DPPH free radical scavenging activity kept on increasing when the concentration of aqueous, ethanol and ethyl acetate extracts of aerial part of *A. paniculata* increased. The maximum DPPH free radical scavenging activity was shown by the ethanol extract, followed by the aqueous extract and at least by the ethyl acetate extract as shown in Figure 3.

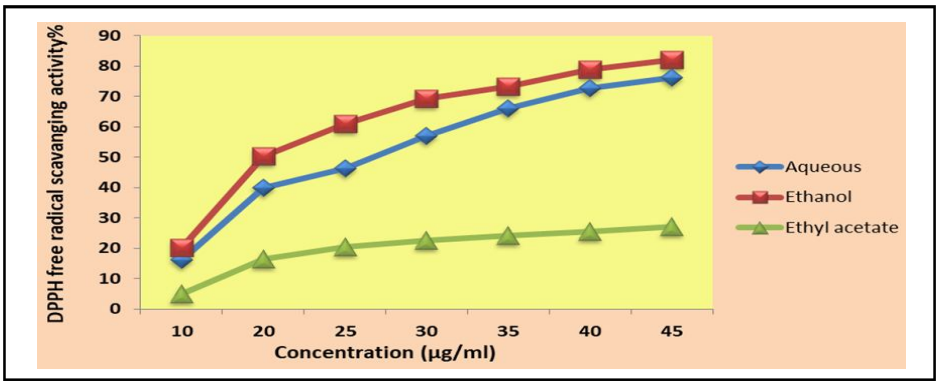

Figure 3: Comparison of DPPH free radical scavenging activity (%) of aerial part of *A. paniculata* in aqueous, ethanol and ethyl acetate extract at different concentration levels.

Furthermore, the lowest IC_{50} value of the ethanol extract was 20.15 µg/ml, followed by 25.47 µg/ml in the aqueous extract and 82.02 µg/ml in the ethyl acetate extract (Table 2). It was thus shown that the extract prepared in ethanol had the highest DPPH free radical scavenging activity, followed by the aqueous extract and the ethyl acetate of the aerial fraction. Ascorbic acid is used as standard for comparing antioxidant activity of plant extract. It is found that ascorbic acid's ability to scavenge free radicals was 92.51% at a concentration of 30 µg/ml, followed by values of 88.21, 74.18, 61.42, 48.12, and 28.54% at concentrations of 25, 20, 15, and 10 µg/ml, respectively. The IC_{50} for ascorbic acid was 11.33 µg/ml. Therefore, ascorbic acid is a better antioxidant than the plant extracts.

Table 2: IC_{50} (µg/ml) values among different solvent (aqueous, ethanol and ethyl acetate) extracts of *A. paniculata* aerial part

	***A. paniculata* aerial part extracts**		
	Aqueous	**Ethanol**	**Ethyl acetate**
Quadratic regression equation	$y = -0.020x^2 + 2.893x - 10.71R^2 = 0.996$	$y = -0.045x^2 + 4.228x - 16.98R^2 = 0.997$	$y = -0.017x^2 + 1.579x - 8.504R^2 = 0.993$
IC_{50} (µg/ml)	25.475	20.15	82.02

4. Discussion

Recent studies have shown that the majority of physiological deficits, tissue damage, pathological processes, and diseases that impact people are caused by free radicals, commonly known as reactive oxygen species (Tawaha *et al*., 2007). In the medical and biological sciences, antioxidants are becoming recognized as being advantageous for health and disease. The primary cause of the antioxidant effect is related to the redox properties of phenolic compounds, which promote the breakdown of peroxides and enable them to bind and neutralise free radicals. Traditionally, the leaves of this plant have been utilized in raw and decoction form (Ito *et al*., 1983).

The moisture content, ash content, crude fibre content, crude protein content, total carbohydrates, alkaloid content, tannin content, total phenolics, total flavonoids and antioxidant activity of *A. paniculata* aerial part were measured in the current study. Kaskoos and Ahamad (2014) showed that the moisture content of the aerial part of *A. paniculata* is about 4.73 ± 1.64%. They also estimated the aerial ash content of *A. paniculate* to be 11.64%. Since the location is different, the ash content of the air part is also different. Therefore, the data obtained is consistent. The crude protein content of the air portion was estimated to be 18.32% by Ali *et al*. (2019). Ali *et al*. (2019) estimated the crude fiber content in leaves of *A. paniculata* to be 6.57%. Abasiekong and Osabor (2017) also reported that the crude fiber content of stems and leaves was 12.40 % and 6.22 %, respectively. Abasiekong and Osabor (2017) estimated the crude fat content of *A. paniculata* stems and leaves to be 1.44% and 9.71%, respectively. The crude fat content of the air portion was estimated to be 12.04% by Ali *et al*. (2019). Abasiekong and Osabor (2017) estimated the total carbohydrates content in stems and leaves 72.15 and 65.04%, respectively. Hence, the determined data is in agreement with earlier findings. Agarwal and Varma (2014) also estimated the total tannin content of the whole plant to be 5.39% and the total alkaloid content of the whole plant to be 2.28%. Sangeetha *et al*. (2014) also reported the total phenolic content in *A. paniculata* using different solvents and found 0.33 ± 0.02 mg GAE/g of the total phenolic content in ethyl acetate extract. Sani *et al*. (2016) reported the total flavonoid content of 0.43 mg of QE/g in aqueous extract. Kataky and Handique (2010) extracted *A. paniculata* using different solvents to determine the yield obtained and the most suitable extract for antioxidant activity. The results showed that the ethanol extract gave the highest percentage (80.59%), followed by the aqueous extract (69.51%) and the ethyl acetate showed the least percentage of free radical scavenging activity (28.54%). As a result, it was determined that the data were consistent with those of other researchers.

5. Conclusion

As extraction plays a great role with different solvents of different polarities and thus exhibited different bioactivity. It is concluded that extract prepared in aqueous solvent has highest amount of total phenolics and flavonoids followed by ethanol and ethyl acetate solvent. Hence, aqueous extract is found to be best among three solvents for phenolics and flavonoids whereas in case of antioxidant activity, ethanol solvent perform the best activity followed by aqueous and ethyl acetate solvent.

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Conflict of interest

The authors declare no conflict of interest relevant to this article.

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