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Full Length Original Research Paper

A detailed analysis of the Antioxidant activity of the Medicinal Plant *Andrographis paniculata*

Sundaramoorthy Sangeetha[1*]

Rastogi Archit[1]

Sathiavelu Mythili[1]

Arunachalam Sathiavelu[1]

[1]School of Bio-Sciences and Technology, VIT University, Vellore – 632 014.

Corresponding Authors:
E-mail:
sangeesundar@gmail.com

Abstract:
Extreme urbanization has led to a drastic change in lifestyles. These new lifestyles expose us to a number of oxidants which are very harmful. This study aimed at identifying the antioxidant properties of the medicinal plant *Andrographis paniculata.* Sequencing extracts of the plant were prepared in n-Hexane, Ethyl Acetate, Chloroform and Methanol respectively. These were then tested for their total phenolic content, free radical scavenging activity (DPPH), hydrogen peroxide radical scavenging activity, β-carotene bleaching activity and total reducing power. *A. paniculata* was found to have strong antioxidant properties. All the studied indicated that the methanolic extract is the most potent antioxidant. This holds great promise for the use of *A. paniculata*as a source of strong antioxidant compounds.

Keywords: *Andrographis paniculata,* Antioxidant Activity, DPPH, Total Reducing Power, β-Carotene Bleaching Assay, Hydrogen Peroxide Radical Scavenging Assay, Phytochemistry

Introduction

Andrographis paniculata, known as *Kalmegh*in the Indian system of Ayurveda, is a widely used home remedy for the common cold. The plant is native to India and Sri Lanka and belongs to the family *Acanthaceae*. The effectiveness of this herb has been widely recognized and its demand is on the rise. [1,2]The major active compound in the plant is Andrographolide. [3] This is often called a wondrous compound which possesses anti-inflammatory, anti-cancer, antiviral and even anti-malarial properties. [4-8]The present study is aimed at understanding the plant's role and value as an anti-oxidant. This study is a continuation of our earlier work. [9]Anti-oxidants are gaining importance due to the increase in the reactive oxygen species generated in our cells due to modern lifestyle. [10]Along with being an anti-diabetic drug, if the extracts are found to be potent anti-oxidants, their pharmacological value would greatly increase.

Materials and methods

Chemicals and reagents: All the chemicals, reagents, kits and solvents used in this study were of analytical grade and procured locally.

Plant material and extract powder: Fresh leaves of the plant were collected from Gudiyattham District, Tamil Nadu, India. Plant material was shade dried and ground into a powder. Sequencing extracts were prepared by successive maceration of the powder (10g) at room temperature with 100ml of various solvents on an orbital shaker for 2 days. The solvents used were n-Hexane, Ethyl Acetate, Chloroform and Methanol respectively. The final extracts obtained were filtered and the filtrates were subjected to lyophilisation (Shimadzu Analytical (India) Pvt Ltd,

Mumbai, India) to obtain powdered extracts which were used for the following assays.

Antioxidant Activity: 1ml of four concentrations (25, 50, 75 and 100mg/ml) of each extract of the plant dissolved in methanol was added to 1ml of DPPH (0.16mM in methanol). 1ml DPPH in methanol with 1ml pure methanol was used as a control. The reaction mixtures were mixed and incubated in the dark at room temperature for 30minutes. Their absorbance was then read at 517nm. [11]The DPPH scavenging ability of plant extracts was calculated using the following equation:

$$Antioxidant\ Activity(\%) = \left(\frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}\right) \times 100$$

Total Phenolic Content: 1ml aliquots of extracts (100 mg/l) were added to a volumetric flask containing 9 ml of water. 1 ml of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 min at 25°C. The absorbance against reagent blank was determined at 750 nm. A reagent blank was prepared and the amount of phenolic compound in the extract was determined from the standard curve. The total phenolic content of the plant was then calculated based on the equation below and expressed as mg gallic acid equivalent (GAE)/g fresh weight.[12]

$$C = \frac{c \times m}{V}$$

Where C = total content of phenolic compound in gallic acid equivalent (GAE)/g, c = the concentration of gallic acid established from the calibration curve (µg/ml), V= volume of extract (ml), and m = weight of the crude plant extract (g).

Hydrogen Peroxide Radical Scavenging Activity: The hydrogen peroxide (H_2O_2) radical scavenging activity was determined by the Ruch *et al* method.1 ml of extract at four concentrations 25, 50, 75,100 mg/ml was taken in 4 test tubes. 0.6 ml of hydrogen peroxide (40mM) was added with phosphate buffer. The samples were incubated at 30^{0}C for 10 minutes. After incubation, absorbance was determined at 230 nm against phosphate buffer as a blank. [13]This was done in replicate. The percentage H_2O_2scavenged was calculated using the formula:

$$\%Scavenged(H_2O_2) = \left(\frac{A_1 - A_2}{A_1}\right) \times 100$$

Where A_1= absorbance of the H_2O_2 without extract.A_2= absorbance of the sample with extract.

β- carotene bleaching activity: 3ml of β-carotene solution (5mg of β carotene/50ml of chloroform) was added to 40mg of linoleic acid and 400mg of Tween 20. Then, the mixture was mixed well and dried. To the dried mixture, 100ml of distilled water was added in order to form β-carotene linoleic acid emulsion. 1ml of solvent extract was taken in 4 test tubes and 1.5ml of emulsion was added. Then, the mixture was incubated in water bath at 50ºC for 60 minutes. This was done in replicate. Finally, the absorbance was read at 470nm.[14]This was done in triplicates. Antioxidant activity (AA %) of *A. paniculata* extract was calculated by using the formula:

$$\%AA = 100 \times \left(\frac{DR_C - DR_S}{DR_C}\right)$$

Where DR_C= Degradation rate of the control, DR_S= degradation rate of the sample ((a/b)/60) a=initial absorbance of the sample. b=absorbance after 60 min of incubation.

Reducing power: The ability of extracts to reduce iron (III) was determined. Four concentrations (25, 50, 75 and 100 mg/ml) of *A. paniculata* solvent extracts were mixed with 2.5ml of phosphate

buffer and 2.5 ml of potassium ferricyanide. Then, the mixture was incubated at 50^{0}C for 30 minutes. 2.5 ml of trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant solution was taken and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride. L-ascorbic acid was used as standard. Finally, the absorbance of all samples was measured at 700 nm. This was done in triplicates.[15]

Statistical Analysis

Statistical analyses were carried out using Microsoft Excel 2010. The standard errors in all the analyses were found to be less than or equal to 5% of the mean values.

Results and Discussion

Total Phenolic Content: The results for the total phenolic content are given in Table 1. As can be inferred from the table, the methanolic extract had the highest total phenolic content. This further implies that the methanolic extract contains the maximum number of tannins and flavonoids, since these are phenolic compounds.

Table 1: This table gives the total phenolic content of the 4 extracts in Gallic Acid Equivalents/gram.

TOTAL PHENOLIC CONTENT	
Extract	**Total Phenolics (GAE/g)**
n-Hexane	0.35±0.0175
Ethyl Acetate	0.33±0.0165
Chloroform	0.51±0.0255
Methanol	0.82±0.0410

The values represent Mean±SEM for sets of three values.

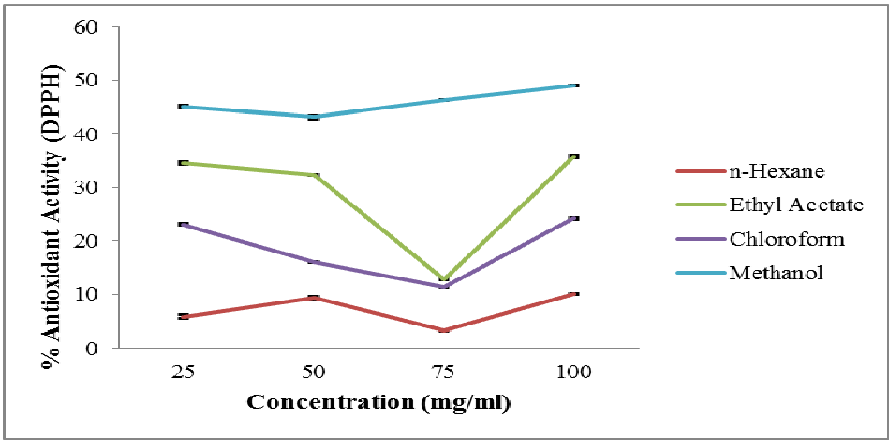
Antioxidant Activity: The results for the antioxidant activity are given in Table 2. The antioxidant activity for the methanolic extract was found to be the highest. In all cases except the methanolic extract, the antioxidant activity decreased till a concentration of 75 mg/ml, after which, a sharp increase was seen at 100 mg/ml. The antioxidant activity was found to be highest at a concentration of 100 mg/ml. The antioxidant activity increased with concentration incase of the methanolic extract. These results have been graphically represented in Figure 1. The higher antioxidant activity of methanolic extract can be attributed to its higher phenolic compound content. This is because flavonoids, the primary compounds responsible for antioxidant activity of plants are phenolic compounds. [16]

Table 2: This table gives the % antioxidant activity of the 4 extracts at 4 concentrations.

Antioxidant Activity				
Concentration(mg/ml)::Extract	**Hexane**	**Ethyl Acetate**	**Chloroform**	**Methanol**
25	5.84±0.34	34.6±0.28	23.06±0.18	45.02±0.24
50	9.32±0.28	32.4±0.14	16.04±0.12	43.14±0.29
75	3.26±0.14	12.8±0.19	11.34±0.16	46.28±0.03
100	10.09±0.16	35.8±0.24	24.26±0.18	49.04±0.12

The values represent Mean±SEM(%) for sets of three values.

Figure 1: This graphgives the % antioxidant activity of the 4 extracts at 4 concentrations. Values are Mean±SEM for a set of three values with their standard errors indicated by vertical bars.



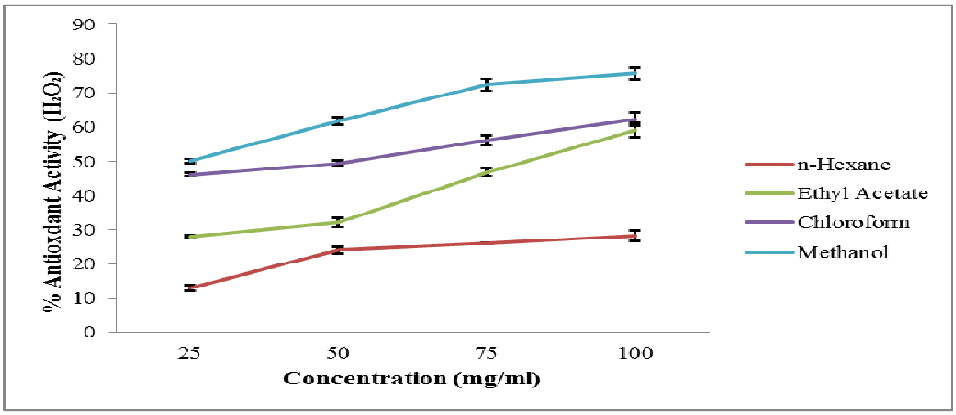
Hydrogen Peroxide Radical Scavenging Activity: The results of the H_2O_2 radical scavenging activity are given in Table 3. The scavenging activity of the methanolic extract was found to be the highest, followed by chloroformic and ethyl acetate, respectively. This has special significance due to the fact that H_2O_2 is a potent oxidant. It has a strong tendency to oxidize DNA in the cells, causing mutations. Reactive agents of H_2O_2 may sometimes even cause cell death due to the generation of free hydroxyl radicals inside the cell. The H_2O_2 radical scavenging activity is attributed to primary antioxidants, which in case of plants would be phenolics. [17] Thus, the higher scavenging activity of the methanolic extract is most likely due to this. The results are graphically depicted in Figure 2.

Table 3: This table gives the % H_2O_2radical scavenging activity of the 4 extracts at 4 concentrations.

H_2O_2 Radical Scavenging Activity				
Concentration(mg/ml)::Extract	**Hexane**	**Ethyl Acetate**	**Chloroform**	**Methanol**
25	13.02±0.86	28.1±0.24	46.1±0.42	50.1±0.68
50	24.08±0.92	32.2±1.4	49.5±0.85	61.8±1.2
75	26.09±0.24	46.8±1.26	56.1±1.36	72.4±1.68
100	28.23±1.63	59.1±2.01	62.4±1.88	75.6±1.78

The values represent Mean ± SEM(%) for sets of three values.

Figure 2: This graphgives the % radical scavenginx activity of the 4 extracts at 4 concentrations. Values are Mean±SEM for a set of three values with their standard errors indicated by vertical bars.



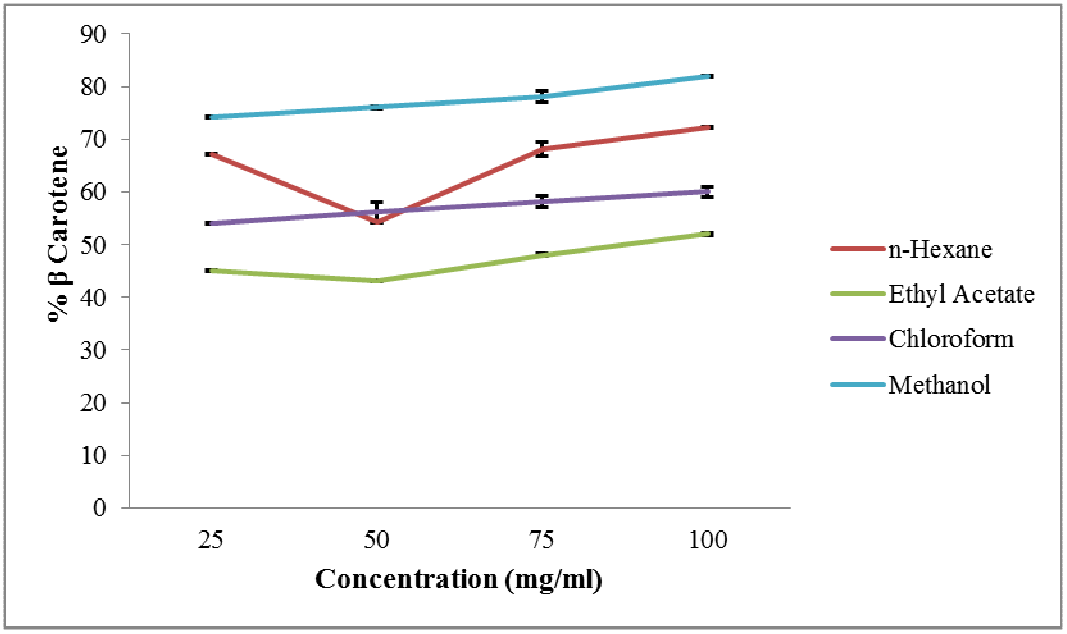
β–carotene bleaching activity: The results of the β–carotene bleaching activity are given in Table 4. β–carotene bleaching activity is again an indirect measure of the antioxidant activity of a plant extract. β–carotene gets bleached by hydroperoxides like H_2O_2. [18] In the presence of antioxidants, this bleaching is greatly reduced due to the neutralizing effect of the antioxidants on the hydroperoxides. Again, the methanolic extract showed the lowest amount of β–carotene, and thus, the highest concentration of antioxidants. This can be correlated to the results of the antioxidant activity and total phenolic content. The results are graphically depicted in Figure 3.

Table 4: This table gives the % β–carotene bleaching activity of the 4 extracts

β–Carotene Bleaching Activity				
Concentration(mg/ml)::Extract	**Hexane**	**Ethyl Acetate**	**Chloroform**	**Methanol**
25	67.24±0.14	45.03±0.16	54.00±0.18	74.08±0.12
50	54.32±0.18	43.12±0.18	56.13±2.00	76.02±0.24
75	68.14±1.29	48.07±0.29	58.12±1.04	78.04±1.02
100	72.32±0.14	52.01±0.24	60.03±1.02	82.06±0.14

The values represent Mean±SEM(%) for sets of three values

Figure 3: This graphgives the % β–carotene bleaching activityof the 4 extracts at 4 concentrations. Values are Mean±SEM for a set of three values with their standard errors indicated by vertical bars.



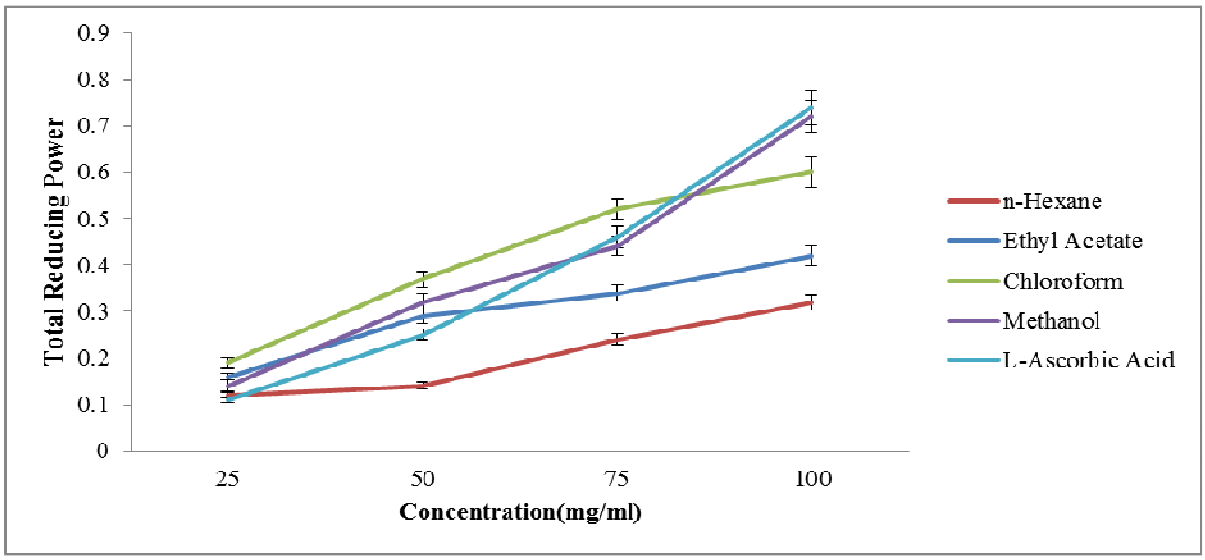
Reducing Power: The ferric reducing assay measures the ability of an antioxidant to reduce a reactive oxygen species against that species' oxidative power.[19]This is important to make the reactive oxygen species more stable and unreactive. The results are tabulated in Table 5 and graphically represented in Figure 4. As can be observed from the graph, the methanolic extract showed a reducing power nearly equal to that of L–Ascorbic Acid. The chloroformic extract showed a very good reducing power at lower concentrations. However, at higher concentrations, its reducing power was lesser than that of the methanolic extracts.

Table 5: This table gives total reducing power of the 4 extracts at 4 concentrations.

Reducing Power					
Concentration(mg/ml)::Extract	Hexane	Ethyl Acetate	Chloroform	Methanol	Ascorbic Acid
25	0.12±0.006	0.16±0.008	0.24±0.012	0.14±0.007	0.11±0.005
50	0.14±0.007	0.29±0.014	0.34±0.017	0.32±0.016	0.25±0.012
75	0.24±0.012	0.34±0.017	0.42±0.021	0.44±0.022	0.46±0.023
100	0.32±0.016	0.42±0.021	0.68±0.034	0.72±0.036	0.74±0.037

The values represent Mean±SEM for sets of three values.

Figure 4: This graphgives the total reducing power of the 4 extracts at 4 concentrations. Values are Mean±SEM for a set of three values with their standard errors indicated by vertical bars.



Conclusion

Based on the above experiments, we can conclude that methanolic extracts of *A. paniculata* are the most effective as antioxidants. These could find potential application in today's urban lifestyle which increases our exposure to various harmful oxidants. These findings indicate that in addition to its anti-diabetic effect, *A. paniculata* has a potent antioxidant activity.

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Conflict of Interest

The authors would like to declare that they have no conflict of interest.

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