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PHYTOCHEMICAL AND INVITRO-ANTIOXIDANT ACTIVITIES OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA*

Rajendrakumar T[1*], Suguna Rao[2], Satyanarayana, M.L.[3], Narayanaswamy, H.D.[4] and Byregowda, S.M.[5]

[1]Assistant Professor, Department of Veterinary Pathology, Veterinary College, Bidar.
[2]Professor, [3]Professor and Head, Department of Veterinary Pathology, Veterinary College, Bengaluru. [4]Vice-chancellor, KVAFSU, Bidar.[5]Director, IAH &VB, KVAFSU, Bengaluru.
E-mail: drrajendra4428@gmail.com (**Corresponding author*)

Abstract: Oxidative stress is an imbalance between free radicals and antioxidant in the body. Hence the application of external source of phyto-antioxidants can assist in coping the oxidative stress. Thus, the search for effective, nontoxic natural compound with antioxidant activity has been intensified. *Andrographis paniculata* is traditionally used in Indian system of medicine to cure the diseases. This study outlined to evaluate phytochemical analysis and invitro antioxidant potential of ethanolic extract of *Andrographis paniculata*. The phytochemical analysis revealed the presence of alkaloids, terpenoids, phenolic compounds, tannin, steroids etc. The free radical scavenging activity was comparable with standard ascorbic acid (Vit-C) in invitro antioxidant activities. Therefore, the *Andrographis paniculata* considered as very good antioxidant source.

Keywords: *Andrographis paniculata*, oxidative stress, phytochemicals, free radical scavenging activity.

1. INTRODUCTION

Medicinal plants are integral part of human life. Th indigenous medicinal plants and plant derived drugs are the potential source of alternative medicine (Hossain *et al.*, 2014). Various phytochemical components from medicinal plants are known to be responsible for antioxidant and free radical scavenging activity (Patel *et al.*, 2010). Antioxidants have been reported to prevent oxidative damage by free raadicals and reactive oxygen species (ROS) and prevent the occurrence of diseases (Sangeetha and Venkatalakshmi, 2017). *Andrographis paniculata* is one such medicinal herb and it is widely used as traditional medicine in China, India and Taiwan (Chen *et al.*,2013). It is commonly known as King of Bitters and it come from family Acanthaceae (Wasman *et al.*, 2011). It is used as a hepatoprotective, antioxidant, anti-inflammatory, antipyretic, antiviral, antibacterial and antidiabetic activity (Trivedi and Rawal, 2001). Hence, in the present study, attempt is made to evaluate the phytochemicals and antioxidant activity.

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2. MATERIALS AND METHODS

2.1 Plant extract and Chemicals: Dried whole plant ethanolic extract powder of *Andrographis paniculata* was obtained from Himalaya herbal pvt Ltd. Bangalore, India and it was preserved in a sealed vial at 4˚C until tested and analyzed. All other chemicals and reagents used for the study were procured from local sources and were of analytical grade.

2.2 Preliminary phytochemical tests for the *Andrographis paniculata* extract: A qualitative phytochemical screening of the ethanolic extract of the *Andrographis paniculata* to detect the presence of essential phytoconstituents, such as alkaloid, tannin, saponin, flavonoid, anthraquinone glycoside, steroids/terpenes, glycosides, proteins, carbohydrates and phenol, was carried out using standard biochemical procedures.

2.2.1 Test for Carbohydrates: Benedict test

2ml of the extract sample mixed with 2ml of the Benedict's reagent and heated in a boiling water bath for 10 minutes. The change in colour to yellow, green to red indicates the presence of reducing sugars.

2.2.2 Test for proteins: Biuret test

3 ml of the extract sample mixed with 1ml of 4% w/v sodium hydroxide and 1ml 1% copper sulphate. The change in colour of the solution to violet or pink indicates the presence of the proteins.

2.2.3 Test for alkaloids

Wagner's test: To a few ml of filtrate, few drops of Wagner's reagent was added on the side of the test tube. A reddish – brown precipitate confirmed the test as positive.

Dragendroff's test: Dragendroff's reagent was added in a test tube containing 2 to 3 ml of extract filtrate. A prominent yellow precipitate indicated the test as positive

2.2.4 Test for Phenols

Ferric chloride test: Two to three drops of 1% Ferric chloride solution was added into 1 mL of extract sample. Phenolic compounds produce a deep violet color or black precipitate with ferric ions.

2.2.5 Test for flavonoids: Alkaline reagent test

2ml of the plant extract was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour which becomes colourless on addition of dilute hydrochloric acid, indicate the presence of flavonoids

2.2.6 Test for terpinoids

Salkowkis Test: The Sample was separately shaken with chloroform (2 mL) followed by the addition of concentrated sulphuric acid (2 mL) along the side of the test tube, a reddish-brown coloration of the interface indicates the presence of terpenoid.

2.2.7 Test for tannins

The sample was stirred with distilled water (10 mL) and then filtered. A few drops of 5% ferric chloride were then added. Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins.

2.2.8 Test for Saponins

The extract sample (2gm) was shaken vigorously with distilled water (20 mL) in a test tube. The formation of frothing indicates the presence of saponins.

2.2.9 Test for steroids: Libermann Burchard test

1ml of the plant extract was treated with few drops of chloroform, acetic anhydride and concentric sulphuric acid and observed the formation dark pink or red colour indicates the positive for steroids.

2.2.10 Test for Anthraquinone glycoside

To the extract solution (1 mL), 5% sulphuric acid (1 mL) was added. The mixture was boiled in a water bath and then filtered. Filtrate was then shaken with equal volume of chloroform and kept to stand for 5 min. Then lower layer of chloroform was shaken with half of its volume with dilute ammonia. The formation of rose pink to red color of the ammonical layer gives indication of anthraquinone glycosides.

2.3 In vitro antioxidant activity

2.3.1 Determination of DPPH scavenging activity

The antioxidant activity based on scavenging of stable DPPH free radical, was determined as described previously (Mensor *et al.,* 2001). An aliquot of 0.5 ml of each fraction doses (20, 40, 60, 80, 100 and 120 µg/ml) test solution in methanol was mixed with each 2.5 ml of 0.5 mM methanol solution of DPPH. The mixture was shaken well and incubated for 10 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control and it was mixed in each similar concentration and doses in DPPH as a test solution. All tests and analyses were run in triplicates and the results obtained were averaged. DPPH free radical scavenging ability (%) was calculated by using the formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.3.2 Determination of phosphor–molybdenum scavenging activity

The antioxidant activity of the extract was determined by the phosphor–molybdenum method as described previously (Prieto *et al.,* 1999). The extract (0.3 ml) of each fractions dose (20, 40, 60, 80, 100 and 120 µg/ml) test solution in methanol of was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95°C, for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer against blank. Methanol (0.3 ml) in place of extract was used as the blank. Ascorbic acid was used as a positive control and it was mixed in each similar concentration and doses as a test solution. All tests and analyses were run in triplicates and the results obtained were averaged. Phosphor–molybdenum free radical scavenging ability (%) was calculated by using the formula.

$$\% \text{ inhibition} = \frac{\text{Absorbance of control- Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Phytochemical analysis

The results of our study showed that, ethanolic extract of the *Andrographis paniculata* was found to possess various phytochemicals or polyphenols such as flavonoids, tannins, phenolics, saponins, steroids, carbohydrates, proteins, terpenoids and alkaloids (Table. 1). This result is in agreement with the study carried out by Bajpai *et al.* (2014); Dwivedi et al., (2015); Adegboyega and Oyewole (2015); Sitara *et al.,* (2016) and Rajalakshmi and Cathrine (2016) who reported the presence of carbohydrates, proteins, alkaloids, flavonoids, terpenoids, phenolics, tannins, steroids and saponins. Herbal plants have gained powerful attention due to its effective role in chemo-therapeutic agents. Their prolific effects are mainly due to their phytoconstituents (Kulyal *et al.,* 2010). The various phytochemical compounds detected are known to have beneficial importance in the medical science (Okeke *et al.*, 2001). In recent years, several researchers have reported that phytochemicals including alkaloids, glycosides, terpenoids, saponin, phenols and steroids have enormous antioxidant and free radical scavenging activities (Farhan *et al*., 2012).

Table-1: Phytochemical constituents of ethanolic extract of *Andrographis paniculata*

S.No	Phytochemical tests	Results
1	Test for carbohydrates	+ve
2	Test for proteins	+ve
3	Test for alkaloids a. Wagner test b. Drgendroff test	 +ve +ve
4	Test for phenolic compounds	+ve
5	Flavonoids	+ve
6	Terpenoids	+ve
7	Tannins	+ve
8	Saponins	+ve
9	Steroids	+ve
10	Anthroquinone glycoside	-ve

3.2 Invitro antioxidant activity

3.2.1 DPPH scavenging activity

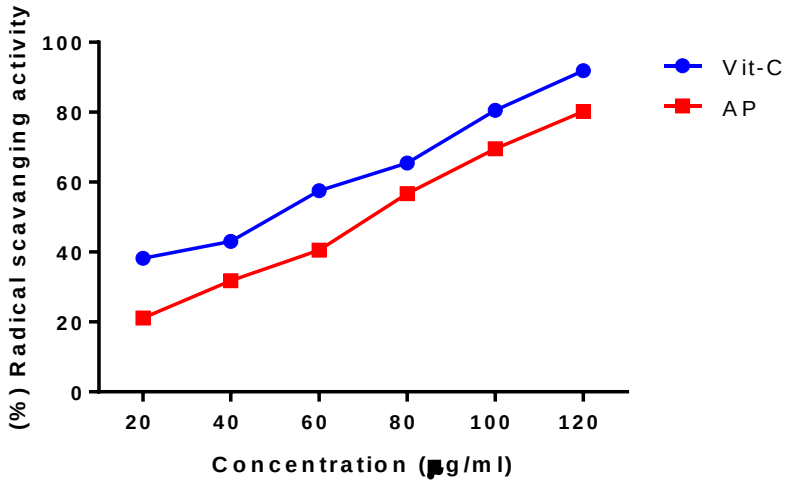
DPPH is one of the free radical widely used for testing preliminary radical scavenging activity of a plant extract. In the present study ethanolic extract of AP showed potential free radical scavenging activity in dose dependent manner and statistically significant ($p<0.05$) along with standard vit-C (Table-2, Figure 1). The scavenging effect of the ethanolic extract of AP and standard vit-C at the concentration of 100 µg/mL were 69.52±0.52 % and 80.53±0.13% respectively.

Table-2: DPPH Radical scavenging activity (%)

Concentration (µg/mL)	Extract	Vit-C
20	21.13 ± 0.50^{ax}	38.18 ± 0.47^{ay}
40	31.81 ± 1.56^{bx}	43.06 ± 0.31^{by}
60	40.56 ± 0.23^{cx}	57.55 ± 0.62^{cy}
80	56.69 ± 0.25^{dx}	65.47 ± 0.26^{dy}
100	69.52 ± 0.52^{ex}	80.53 ± 0.13^{ey}
120	80.17 ± 0.42^{fx}	91.84 ± 0.40^{fy}

Values with different superscripts in a row and column vary significantly at $p<0.05$

Figure-1: DPPH Radical scavenging activity (%)



The DPPH radical is a stable free radical, which has been widely used as sensitive and rapid tool to estimate free radical scavenging activity of both hydrophilic and lipophilic antioxidants (Archana *et al.,* 2005). A freshly prepared DPPH solution exhibits a deep purple colour generally fades/disappears when an antioxidant present in the medium. Thus, antioxidant molecule can quench DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2,2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine) resulting in a decreasing absorbance at 518 nm (Yamaguchi *et al.*, 2002). In the present study, the scavenging effects of the extract and standard vit-C is in increasing trend with increasing concentration of plant extract and Vit-C. The results were concentration dependent and similar findings on DPPH radical scavenging activities of plant extracts have been previously observed (Farhan et al. 2012; Amari et al. 2014).

3.2. 2. Phosphor–molybdenum scavenging activity

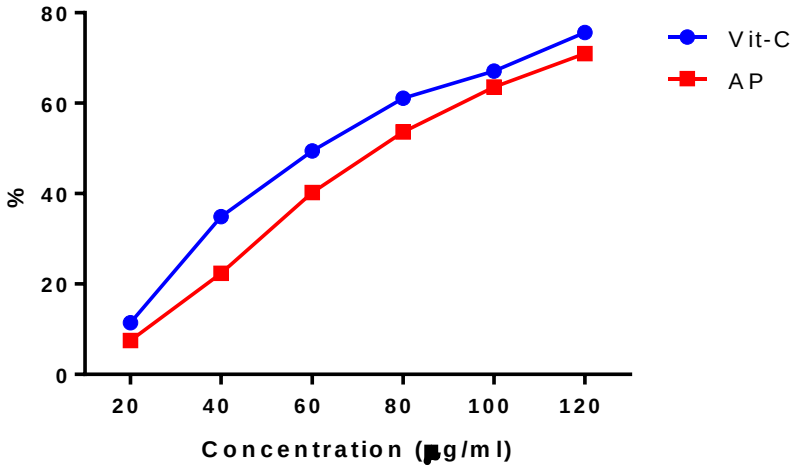
The invitro evaluation of the AP extract for its antioxidant potential revealed that the scavenging potential of the extract and standard vit-C for Phosphomolybdenum radical is in increasing trend with increasing concentration of the extract and standard vit-C (Table 3 Figure 2) and statistically significant ($p<0.05$). The scavenging effect of the ethanolic extract of AP and standard vit-C at the concentration of 100 µg/mL were 63.60±0.64 % and 67.13±0.54 % respectively.

Table-3: Phosphomolybdenum total antioxidant activity (%)

Concentration(µg/mL)	Extract	Vit-C
20	7.46 ± 0.35^{ax}	11.40 ± 0.41^{ay}
40	22.40 ± 0.23^{bx}	34.86 ± 0.50^{by}
60	40.26 ± 0.29^{cx}	49.46 ± 0.30^{cy}
80	53.60 ± 0.87^{dx}	61.13 ± 0.35^{dy}
100	63.60 ± 0.64^{ex}	67.13 ± 0.54^{ey}
120	70.93 ± 1.04^{fx}	75.16 ± 0.24^{fy}

Values with different superscripts in a row and column vary significantly at $p<0.05$

Figure-2: Phosphomolybdenum total antioxidant activity (%)



PM assay is based on the reduction of the phosphate-Mo (VI) to phosphate Mo (V) by the sample and subsequent formation of a bluish green coloured phosphate/Mo complex at acidic pH. Phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts (Prieto *et al.*, 1999). In the present study, the total antioxidant activity of the extract is in increasing trend with increasing concentration of the extract and standard vit-C. Similar findings on Phosphomolybdenum method for total antioxidant activities of various plant extracts have been previously observed (Yadav, 2015; Phatak and Hendre, 2014; Sangeeta and Venkatlakshmi, 2017).

4. CONCLUSION

On the basis of the results obtained in this study, it is concluded that, the ethanolic extract of *Andrographis paniculata* which contain alkaloids, flavonoids, terpenoids, tannins, saponin, phenols and steroids as essential phytochemicals, exhibited significant antioxidant and free

radical scavenging activities. These findings indicate that *Andrographis paniculata*, can be useful therapeutic agent for prevention of oxidative stress.

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