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Evaluation of *In-vitro* antioxidant activity of leaf extract of *Andrographis paniculata*

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ABSTRACT

The aim of the present investigation was to evaluate the in vitro antioxidant activities of leaf extract of Andrographis paniculata by three different in vitro models such as, Hydroxyl radical scavenging activity**,** FRAP method and total phenol estimation. The leaf extract of Andrographis paniculata was found to more effective in the hydroxyl radical scavenging activity. The IC_{50} values of the leaf extract of Andrographis paniculata and ascorbate were found to be 370µg/ml and 410µg/ml respectively. FRAP method of leaf extract and Ascorbate IC_{50} values were found to be 210 µg/ml and 50 µg/ml. In addition, the leaf extract of Andrographis paniculata was found to contain a noticeable amount of total phenols (5.96mg/g), which play a major role in controlling antioxidants. So, the in-vitro studies clearly showed that the leaf extract of Andrographis paniculata has a significant antioxidant activity. It can be concluded that the free radical scavenging activity of the leaf extract of Andrographis paniculata responsible for the therapeutic properties.

Keywords: Andrographis paniculata, Hydroxyl radical scavenging activity, FRAP method, Estimation of total phenol.

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INTRODUCTION

Oxidant stress, a result of imbalance between the antioxidant defense system and the formation of Reactive Oxygen Species (ROS), may damage life important membrane lipids, proteins, DNA and carbohydrates [1]. It is well known that reactive oxygen species (ROS) are involved in many Pathological disorders such as atherosclerosis and related cardiovascular disease [2-3]. Oxidative stress can result either from low levels of anti oxidants and from an increased production of reactive species [4]. Elevated serum lipid levels, particularly cholesterol along with generation of reactive oxygen species (ROS), play a key role in the development of coronary artery disease and atherosclerosis [5]. Many studies have suggested that these naturally occurring flavonoid compounds exhibit biological activities and show a remarkably with scavenging activity toward chemically generated radicals, thus making them effective in inhibiting oxidation of human low density lipoproteins and preventing various human diseases [6-9]. Andrographis paniculata (Acanthaceae) is one of the most important medicinal plant and has been widely used in Chinese and Ayurvedic medicine for the treatment of gastric disorders, infectious diseases and the common cold [10]. The herb contains diterpenoids, flavonoids and poly phenols as the major bioactive component [11].Hence; the aim of the present investigation was to evaluate the antioxidant activity of leaf extracts of Andrographis paniculata by three in vitro methods.

MATERIALS AND METHODS

Collection and Identification of Plant materials

The leaf parts of Andrographis paniculata were collected from Palyamkottai District of Tamilnadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The leaf parts of Andrographis paniculata, were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with 1:1 mixture of dichloromethane and methanol by cold maceration method. The extract was filtered and the solvent were concentrated by using rotary evaporator [12].

EVALUATION OF ANTIOXIDANT ACTIVITY OF IN VITRO STUDIES:

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves in-vitro generation of hydroxyl radicals using

Fe^{3+}/ascorbate/EDTA/H_2O_2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured. In one of the methods the hydroxyl radicals formed by the oxidation is made to react with Dimethyl Sulphoxide to yield formaldehyde. Formaldehyde formed produces intense yellow colour with Nash reagent (2M ammonium acetate with 0.05m acetic acid and 0.02m acetyl acetone in distilled water). The intensity of yellow colour formed is measured at 412 nm spectrophotometrically against reagent blank. The activity is expressed as % hydroxyl radical scavenging [13].

FRAP Method

FRAP is one of the most rapid test and very useful for routine analysis. The antioxidative activity is estimated by measuring the increase in absorbance caused by the formation of ferrous ions from FRAP reagent containing TPTZ (2, 4, 6–tri) (2-pridyl)–S-triazine) and $FeCl_3 6H_2O$. The absorbance was measured spectrophotometrically at 595nm. Antioxidant activity of plant extracts is reported by this method [14].

Estimation of total phenol

The measurement of total phenol is based on Mallick and Singh (1980), to 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2ºc for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3ml of water to the dried supernatant. To which added 0.5ml of folins phenol reagent and 2ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1mins.The absorbance was measured at 650nm in a spectrophotometer [15].

RESULT AND DISCUSSION

Inhibition of hydroxyl radical anion activity

Free radical scavenging activity of the leaf extract of Andrographis paniculata was determined by hydroxyl radical scavenging method. The leaf extract of Andrographis paniculata was exhibited a maximum hydroxyl radical scavenging activity of 72.71 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 80.44 % at 1000 µg/ml. The IC_{50} of plant extract and standard (Ascorbate) was found to be 370µg/ml and 410µg/ml respectively. The percentage of Hydroxyl radical activity of leaf extract of Andrographis paniculata was tabulated in Table -1.

FRAP method

The reducing ability of the leaf parts of Andrographis paniculata and ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were

presented in Table - 2. The maximum reducing ability at 1000µg/ml for plant extract and ascorbate was found to be 79.66% and 98.07% respectively. The IC_{50} values of plant extract and ascorbate was recorded as 210µg/ml and 50µg/ml respectively.

Table-1: Antioxidant activity of leaf extract of Andrographis paniculata on Hydroxyl radical scavenging activity method

S.No	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	39.14±0.04	35.63±0.07
2	250	47.78±0.08	49.89 ±0.04
3	500	58.52±0.05	59.62±0.02
4	1000	72.71±0.04	80.44±0.04
		IC_{50}=370 µg/ml	**IC_{50}=410µg/ml**

*All values are expressed as mean ± SEM for three determinations

Table-2 Reducing ability of leaf extract of Andrographis paniculata by FRAP method

S.No	Concentration (µg/ml)	% of activity (±SEM)	
		Sample (Methanolic extract)	Standard (Ascorbate)
1	125	39.47±0.04	72.04±0.014
2	250	57.11±0.02	82.05±0.034
3	500	66.84±0.05	86.04±0.026
4	1000	79.66±0.03	98.07±0.041
		IC_{50}= 210 µg/ml	**IC_{50}=50µg/ml**

*All values are expressed as mean ± SEM for three determinations

Total phenol

Table - 3 were depicted the total phenolic content of leaf extract of Andrographis paniculata. The leaf extract of Andrographis paniculata was found to contain a noticeable amount of total phenols (5.96mg/g), which play a major role in controlling antioxidants.

Table 3 The total phenolic content of leaf extract of Andrographis paniculata

S.NO	Extract	Total Phenolic content ±SEM(mg/g Catechol)
1	Leaf extract of Andrographis paniculata	5.96±0.54

*All values are expressed as mean ± SEM for three determinations

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CONCLUSION

The results of the above investigation indicated that the leaf parts of Andrographis paniculata showed strong antioxidant activity. However, Phytochemical screening of leaf parts of Andrographis paniculata showed presence of Triterpenoids, Phenolic compound and flavonoids. So it can be concluded that these components might be involved in the antioxidant activity of Andrographis paniculata.

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