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ANTIOXIDANT ACTIVITY AND DNA CLEAVAGE PROTECTIVITY OF *Andrographis alata* EXTRACT

B. Jothi[1]* and S. Ramya[2]

[1] Assistant Professor Department of Biochemistry, Bhaktavatsalam Memorial College for Women,Chennai-80 ,
[2]PG Student Department of Biochemistry, Bhaktavatsalam Memorial College for Women,Chennai-80,

*Corresponding author: jothijune24@gmail.com.

ABSTRACT

Bioflavonoids are a class of plant secondary metabolites, are most common known for their antioxidant activity *in vitro* .*Andrographis alata Nees* is one of the most familiar medicinal plant are known for its availability of flavonoids. It has been suggested that flavonoids can chelate Fe (II) and Fe (III) and prevent free radical production and also intercalate DNA duplex and react with free radicals in order to protect DNA from oxidative demage.The present study was carried to evaluate the free radical scavenging capacity of flavonoid and its effect on linoleic acid peroxidation induced by H_2O_2 UV- photolysis. In addition, its effect on DNA cleavage,induced by H_2O_2 UV- photolysis, was also evaluated..

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Corresponding author
B. Jothi, Department of Biochemistry, Bhaktavatsalam Memorial college for Women,Chennai-80 Tamil Nadu, India.

INTRODUCTION

Free radicals are believed to lie at the heart of the etiology of a number of diseases, inducing certain types of cancer, cardiovascular and neurodegenerative diseases by causing cell demage (Rajagopalan.*et al*, 2005). Hence Antioxidants are used as substances which protect our cell against the effect of free radicals. Flavonoids or bioflavonoidsb (from the Latin words flavus meaning yellow, their colour in nature),are a class of plant secondary metabolities (Vitamin P) present in fruits, vegetables, tea, and red wine. Flavonoids are most commonly known for their antioxidant activity in vitroand the antioxidant abilities of flavonoids in vitro may be stronger than those of vitamin C and E.

Andrographis alata Nees is popularly known as Periyanangai(Tamil),belonging to the family of Acanthaceae is one of the most familiar medicinal plants of Ayurvedha, Homeopathy, Natureopathy, Amchi, Modern, Siddha and Unani. A new flavones glycoside, echioidinin 5-glucoside, aglycone, echioidinin have been isolated from the whole plant of Andrographis alata. *Andrographis alata* has been shown to possess antipyretic, anti-inflammatory, antivenom activity and snake bite. The present work

was framed to study of flavonoid extract from the leaves of Andrographis alata evaluated antioxidant and DNA cleavage protector activity.

METHODOLOGY

Source : ***Andrographis alata*** leaves were used as source.

Preparation of extracts

Organic solvents (methanol) extract of the Andrographis alata leaves were prepared according to the method described by Boaky- Yiadon(1979) with little modifications.

Phytochemical Screening Test of *Andrographis alata* leaves

Test of flavonoids- Two methods were used to test for flavonoids. First, 10% of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. 1 ml of concentrated sulphuric acid was added. A yellow coloration tha disappears on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminium solution were added to a portion of the filtrate. A yellow coloration indicates the presence of flavonoids. The content of flavonoids is determined.

Partial characterization of Thin Layer Chromatography

The Methanol extract of Andrographis alata leaf was loaded on to percoated TLC (60 F2 54) andit was developed using solvent system in the ratio of 1:0.5:0.1 (Hexane, Chloroform and Methanol) visible and the non visible spot given and it is fluorescent with UV light at 360nm.

Total Phenol

The total phenolic content of Andrographis alata leaves methanol extract was determined using the method by Gutfinger(1981).

Superoxide radical scavenging assay

Superoxide radicals were generated via an enzymatic reaction following the procedure of Nisimiki *et al.*, 1972.

DPPH radical scavenging assay

DPPH radical scavenging capacities of ***Andrographis alata*** leaves methanol extract was determined using the method of Santiago et al.1992.

Lipid peroxidation

Different concentrated of ***Andrographis alata***flavonoid extract also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates after 3 h incubation at 37°C.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging capacities of the additives were calculated using the following formula (Shi and others, 1991).

Preparation of DNA

To 10 ml whole blood, add 30 ml lysis buffer and centrifuge at 1200 rpm for 10 min at 4*C. Remove supernatant and add 10 ml lysis buffer and again centrifuge for 10 min at 4*C and Remove supernatant and add 5 ml SE-buffer and centrifuge for 10 min at 4*c(1200 rpm), Add 5 ml SE-buffer and resuspended the pellet add 40 mu I proteinase K 250 mu I 20% SDS, incubate overnight at 37*C in a water bath followed by addition of 5 ml SE-buffer and 10 ml phenol, centrifuge at 3000 rpm for 5 min at 10*C, transfer the supernatant and add 10 ml of chloroform/isoamylalcohol, add 300 mu I 3M sodium acetate(pH 5.2) and 10 ml isopropanol. DNA gets precipitated then measure the DNA concentration in a spectrophotometer and run 200 ng on a 1% agarose gel.

***In vitro* DNA cleavage protector activity**

DNA cleavage protector activity ***Andrographis alata*** leaves flavonoid extract. The experiments were performed in a volume of 20 ml containing 33 mmol/L in bp (7.56 nmol/L) of pBR322 plasmid DNA in 5 mmol/L phosphate buffer contained 10 mmol/L NaCl, pH 7.4, in the presence of different concentrations(200-400mmol/L) ofcatechin,naringin, and rutin. Immediately prior to irradiating the samples with UV light, H2O2 was added to a final concentration of 2.5 mmol/L.

The reaction volumes were held in caps of polyethylene microcentrifuge tubes, which were placed directly on the surface of a transilluminator (8000 mW/cm) at 300 nm. The samples were irradiated for 5 min at room temperature. After irradiation, 4.5 ml of a mixture containing 0.25% bromophenol blue, 0.25% xylelne cyanol FF, and 30% glycerol was added to the irradiated solution. The samples were then analyzed by electrophoresis on a 1% agarose horizontal slab gel in Tris-borate buffer(45 mmol/L Tris-borate), 1 mmol/L reactive substances (TBARS) were determined as described by Stocks (Stocks and Dormandy, 1971). Untreated pBR322 plasmid was included as a control in each run of gel electrophoresis, which was carried out at 1.5 V/cm for 15 h. Gel was stained in ethidium bromide (1 mg/ml; 30 min) and photographed on Polaroid – Type 667 positive land cam.

Statistical analysis

The data were analyzed using one-way ANOVA followed by Student's Newman Keul's (SNK) test was used to assess the statistical significance of each group.

RESULTS

Phytochemical screening

The phytochemical screening of the ***Andrographis alata*** studied showed the presence of flavonoids.

The Partial characterization of methanol leaves extract of *Andrographis alata* by TLC

The methanol leaves extract of ***Andrographis alata*** located on Pre-coated TLC plates (60 F2 54 Merck) and developed with a solvent system of hexane,

chloroform and methanol in the ration of 1:05:0.1 was efficient to extract the antioxidant compound which is used for further studies. The developed plate was viewed under UV 240nm and 360nm.

Total phenolic compound of *Andrographis alata*

The preliminary experiments revealed that 80% acetone was the best solvent for the extraction of phenolics at 60*C for 60 min since it afforded a maximum yield of phenolics(Table.1). The yields leaves of N. nucifera extracts ranged from 12.2% to 20.6%(w/w).

Table: 1 Yield and phenolic content of *Andrographis alata* extracts

Sample	Yield (g/100g)	Total phenolic content (mg/g)
Andrographis alata	16.4±1.6[a]	98.1±2.4[b]

Data are expressed as mean ± standard deviation (n=3) on a fresh weight basis

Superoxide scavenging assay

Andrographis alata flavonoid extracts exhibited potent scavenging activity for superoxide radicals in a concentration dependednt manner. The methanolic purified compound 500 mu g/ml had EC50 values of 556 mu g/ml. Based on their EC50 values; the order of scavenging activity was as follows: 25,50,75 and 100 mu g/ml(Table-2).

Scavanger effect

The free-radical scavenging activity of ***Andrographis alata*** flavonoid extract was also tested by their ability to bleach the stable ABTS radical. This assay provided information on the reactivity of test compounds with a stable free radical. Because of its off electron, ABTS gives a strong absorption band at 517 nm in visible spectroscopy deep violet colour).

Table: 2 Superoxide anion scavenging activity of Andrographis alata flavonoid

Different concentration	(%) of Inhibition
25 (µg/ml)	11.4 ± 58
50 (µg/ml)	62.4 ± 1.3
75 (µg/ml)	94.2 ± 2.1
100 (µg/ml)	118.1 ± 1.1
Ic_{50}	556

All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition (Pearson's correlation analysis). A Mean ± SD.

Table: 3 Scavanger effects of different concentrations of superoxide aniona

Different concentration	(%) of Inhibition
25 (µg/ml)	16.2 ± 8.2
50 (µg/ml)	59.4 ± 4.9
75 (µg/ml)	79 ± 2
100 (µg/ml)	110 ± 2.6
Ic_{50}	542

Results are expressed as percentage inhibited superoxide anion formation with respect to control. Each value represents the mean ± SD

Andrographis alata flavonoid extract showed a significant and dose dependent DPPH quenching capacity, higher concentration of ***Andrographis alata*** flavonoid extract 100 mu g/ml was more efficient lower concentration (Table-3).

Lipoperoxidation

Different concentrated of ***Andrographis alata*** flavonid extract also inhibited the lipid peroxidation induced by ferrous sulfate in egg yolk homogentates. Maximum inhibition was observed with total 100 mu g/ml with EC50 value at 546 and the other fractions were in the same order as described above that is: Total ***Andrographis alata*** flavonoid extract 25, 50,75 and 100 mu g/ml(Table-4).

Table: 4 Inhibition of lipid peroxidation

Different concentration	(%) of Inhibition
25 (µg/ml)	18.3 ± 8.1
50 (µg/ml)	64.7 ± 4.9
75 (µg/ml)	81 ± 1
100 (µg/ml)	96 ± 2.1
Ic_{50}	546

Results are expressed as percentage inhibition of lipoperoxidation with respect to control (2.4 mmol/mg protein). Each value represents the mean ± SD of five experiments.

Site specific hydroxyl radical scavenging assay (with EDTA)

Andrographis alata flavonoid extract 100 mu g/ml was found to be the most potent hydroxyl radical scavenger with EC 50 values of 560 mu g/ml and the 25mu g/ml fractions was the least potent hydroxyl scavenger. Based on their EC50 values the radicals was as follows: Total ***Andrographis alata*** flavonoid extract>25, 50, 75 and 100 mu g/ml(Table-5).

Table : 5 Hydroxyl radical scavenging activity

Different concentration	(%) of Inhibition
25 (µg/ml)	12.2 ± 1.15
50 (µg/ml)	61.3 ± 1.53
75 (µg/ml)	79 ± 3.0
100 (µg/ml)	107 ± 6.2
Ic_{50}	551

All the observations in different groups showed significant (P<0.01) relationship between the concentration and percentage inhibition.

Effects of *Andrographis alata*on the protection of supercoiled DNA

The electrophoretic pattern of DNA after UV-photolysis of H2O2 (2.5 mmol/L) in the absence or presence of the ***Andrographis alata*** flavonoid extract was determined.

Effects of *Andrographis alata* flavonoid extract at deferent concentrations on the protection of supercoiled DNA

DNA derived from pBR322 plasmid showed two bands on agarose gel electrophoresis (lane 1), the faster-moving band corresponding to the native form of supercoiled circular DNA (scDNA) and the slower-moving band being the oopen circular form (ocDNA). The UV irradiation of DNA in the presence of H2O2 (lane 2) caused the cleavage of scDNA to give ocDNA and the linear form (linDNA), indicating that OH generated by UV-photolysis of H2O2 produced DNA strand scission. The presence of all the bioflavonoids under investigation suppressed the formation of linear DNA.

DISCUSSION

Free radicals are involved in the normal physiology of living organisms. They act as a messenger for signal transduction and also affect gene expression (Armario et al., 1990). Besides, FR are also involved in the pathogenesis of several chronic diseases such as neurodegenerative diseases, ageing, rheumatoid arthritis, metabolic diseases like atherosclerosis, diabetes, hypertension, etc. (Misra et al., 1965).

Different fractions of ***A.alata*** and the possible mechanisms by assessing their role on ABTS radicals scavenging activity, lipid peroxidation, superoxide anion removal and hydroxyl radicals trapping potential were investigated. All the fractions of ***A.alata*** exhibited a potent scavenging activity for ABTS radical cations in a concentration dependent showing a direct role in trapping free radicals.

Interestingly, the methanol flavonoid fraction was found to be a very high antioxidant as compared to all other extracts in the polar fraction. ***Alata*** fractions significantly inhibited the degree of lipid peroxidation either due to chelation of Fe or by trapping of the FRs. Tondon and Gupta (2005) have also reported similar antioxidant properties of ***A.alata*** in rats, by using ethanol induced oxidative stress model.

The action of ***A.alata*** is clear indications of its direct reaction with this radical species are being generated through the non-enzymatic method. They exhibited apotent scavenging activity for hydroxyl radical in a concentration dependent in the absence of EDTA indicates the ability of ***A.alata*** to trap OH radicals and also to chelate iron (Haslam, 1996, Halliwell et al., 1987).

CONCLUSION

The present study provides evidence that ***Andrographis alata*** flavonoid extract exhibit interesting antioxidant properties, expressed either by their capcity to scavenge free radicals or their capcity to protect DNA cleavage activity. These effects may be useful in the treatment of pathologies in which free radical production plays a key role.

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