INVITRO ANTIOXIDANT PROPERTIES OF VARIOUS EXTRACTS OF ANDROGRAPHIS ECHIOIDES

Running title: Invitro antioxidant properties of various extracts of *Andrographis echioides.*

ABSTRACT

Background: *Andrographis echioides* is a medicinal herb, used in the treatment of various diseases. It has potential antioxidant properties. The present study plan is to find a novel herbal antidote for oxidative stress from Andrographis echioides.

Objective: The aim of the current study is to analyse the in vitro antioxidant property of aqueous, ethanolic and chloroformic extracts of *Andrographis echioides*.

Materials and methods: DPPH free radical scavenging assay was performed to evaluate the antioxidant potential of *Andrographis echioides*. Experiments were carried out in triplicates and percentage inhibition of DPPH radical scavenging activity was calculated. The data was analysed statistically and the level of significance was considered at the level of $p<0.05$.

Results: There is a dose dependent increase in the percentage of inhibition of DPPH free radical by the extracts. All the three extracts (aqueous, ethanolic and chloroformic extracts) of *Andrographis echioides* showed significant increase in the antioxidant property with concentration ranging from 100-500 µg.

Conclusion: The study concluded that different extracts of *Andrographis echioides* showed effective antioxidant properties and it could protect the biological system against oxidative stress including ageing, cancer, diabetes and cardiovascular disorders.

Key words: Andrographis echioides, free radicals, oxidative stress, antioxidants.

INTRODUCTION

Free radicals are highly reactive unstable molecules which are formed in the body as a byproduct of metabolism. External factors like radiation, exposure to ozone , cigarette smoking, air pollution and industrial chemicals also induce the formation of free radicals(1,2)). The presence of free radicals in the body causes damage to the DNA and other parts of the cell. It induces chain reaction which leads to the formation of many other free radicals thereby increasing the free radical oxidative stress (3)(4)(5). Free radical mediated oxidative stress is considered as the major cause for many diseases like cardiovascular diseases, cataract, arthritis, brain dysfunction, diabetes mellitus, cancer, ageing etc (6)(7)(8)(9).

The risk of these diseases can be reduced by the intake of antioxidants. Antioxidants, also called 'free radical scavengers' are substances which protect the cell from free radicals. It neutralises the free radical by inhibiting the chain reaction. Antioxidants are derived from both natural and synthetic sources. Due to the natural origin, relatively less or no side effects and cost effectiveness, natural antioxidants are mostly preferred over synthetic ones. Vegetables and fruits are rich sources of antioxidants. Many plant extracts exhibiting antioxidant potential have been found by many researchers (10)(11). The present study is directed towards finding an antioxidant of natural origin. The results showed that Andrographis echioides have high antioxidant potential.

Andrographis echioides, commonly known as 'false water willow' is an annual herb widely distributed in the dry tropical regions of India and Sri Lanka (12). Rangaswamy and Rao were the first to examine this plant (13). *Andrographis echioides* is a medically important plant. It is used in the treatment of goitre, liver diseases, fever, fertility problems, bacterial, malarial, fungal, helminthic, diarrhoea and larvicidal disorders. It is also used in the treatment of snake bite and scorpion sting (14). The plant is listed in the Indian materia media as a remedy for the treatment of illness as its juice is used to cure fever (15). Besides antioxidant properties, *Andrographis echioides* also possess anti diabetic, anti ulcer, antipyretic, anti-inflammatory, antidiuretic, antimicrobial, analgesic and hepatoprotective properties (16).

Scientific Classification of *Andrographis echioides*

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Scrophulariales

Family: Acanthaceae

Genus: Andrographis.

Species: echioides.

Common name: False water willow(17)

The current study is to evaluate the in vitro antioxidant activity of aqueous, ethanolic and chloroformic extracts of andrographis echioides.

MATERIALS AND METHODS

Study design

The study was conducted in the Blue lab in Saveetha Dental College, Chennai.

Ethical considerations

Ethical approval for the study was obtained from the Institutional Review Board (IRB), Saveetha Dental College.

Chemicals

All the reagents and chemicals needed for the study was purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA.

Assessment of in vitro antioxidant potential of aqueous, ethanolic and chloroformic extracts of Andrographis echioides.

DPPH radical assay

The DPPH free radical scavenging assay was performed by Liyana Pathirana and Shahidi method [(18)]. 200 µL of 0.1 mM DPPH prepared in methanol was added to 100 µg/ml of the

plant compounds ,with an increase in concentration (100 to 500 µg/ml). The resulting mixture was incubated in the dark, at room temperature for about 15 minutes. Absorbance was observed at 517 nm. BHT was taken as a positive control. The experiment was carried out in triplicates to calculate the percentage inhibition of the DPPH radical scavenging activity.

% Inhibition=((A0 -A1)/A0)*100

Where,

A0is the absorbance of the control and

A1 is the absorbance of the sample.

Statistical analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

RESULTS

The results show that there is a dose-dependent increase in the percentage of inhibition of DPPH free radicals by the extracts with an increase in concentration ranging from 100-500 µg.

Aqueous, ethanolic and chloroformic extracts showed significant increase in antioxidant potential with the increase in their concentration ranging from 100-500 µg. ($p< 0.05$). (Fig 1-3)

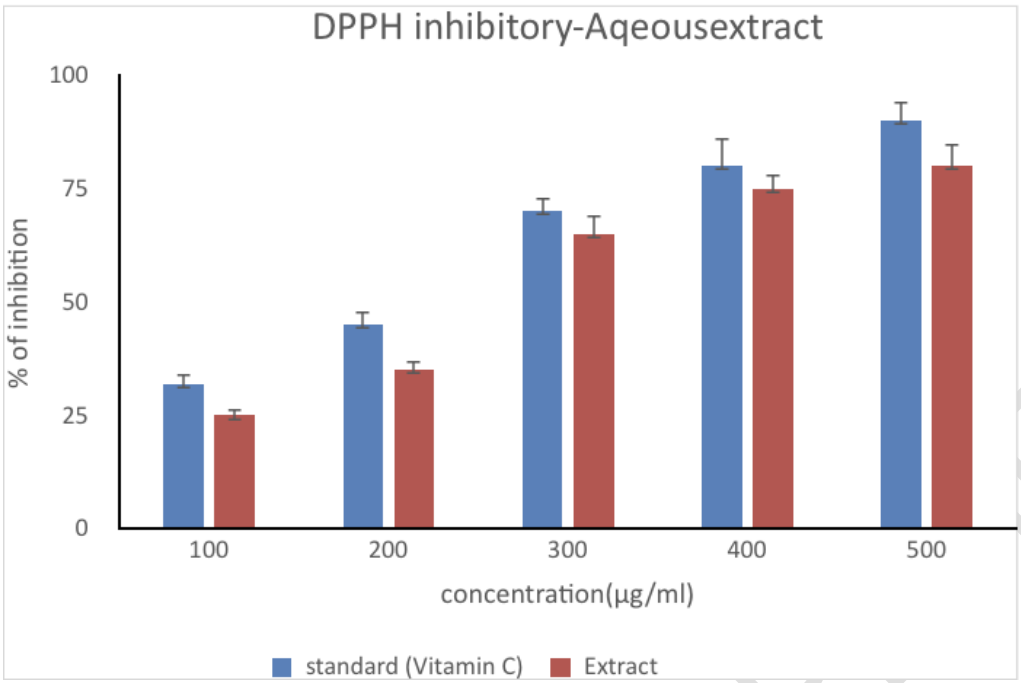

Figure1: Represents bar graph showing DPPH inhibitory activity of aqueous extract of Andrographis echioides. Blue colour bar represents the percentage of inhibition of DPPH by the standard drug (vitamin C) and the red colour bar represents the percentage of inhibition of DPPH by aqueous extract of Andrographis echioides. Each bar represents the mean ± SD of 5 observations. Significance at the levels of $p < 0.05$. a-compared with 100 µg; b-compared with 200 µg; c-compared with 300 µg.

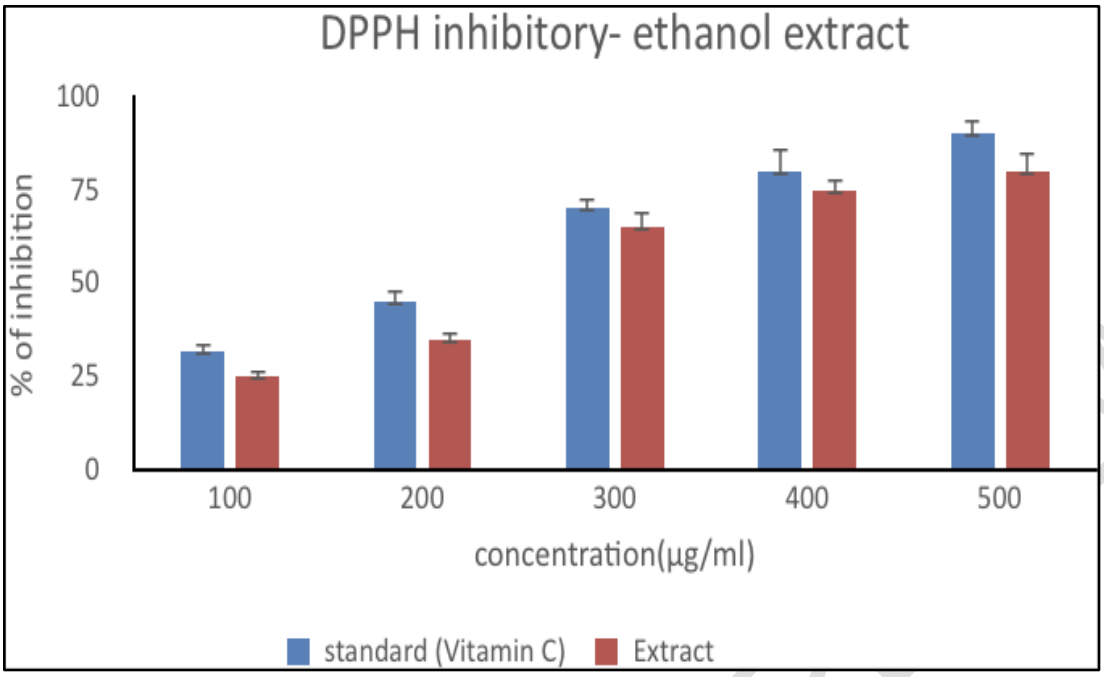

Figure 2: Represents bar graph depicting the DPPH inhibitory activity of ethanolic extract of Andrographis echioides. Blue colour bar represents the percentage of inhibition of DPPH by the standard drug (vitamin C) and the red colour bar represents the percentage of inhibition of DPPH by ethanolic extract of Andrographis echioides. Each bar represents the mean ± SD of 5 observations. Significance at the levels of $P < 0.05$.a-compared with 100 µg; b-compared with 200 µg.

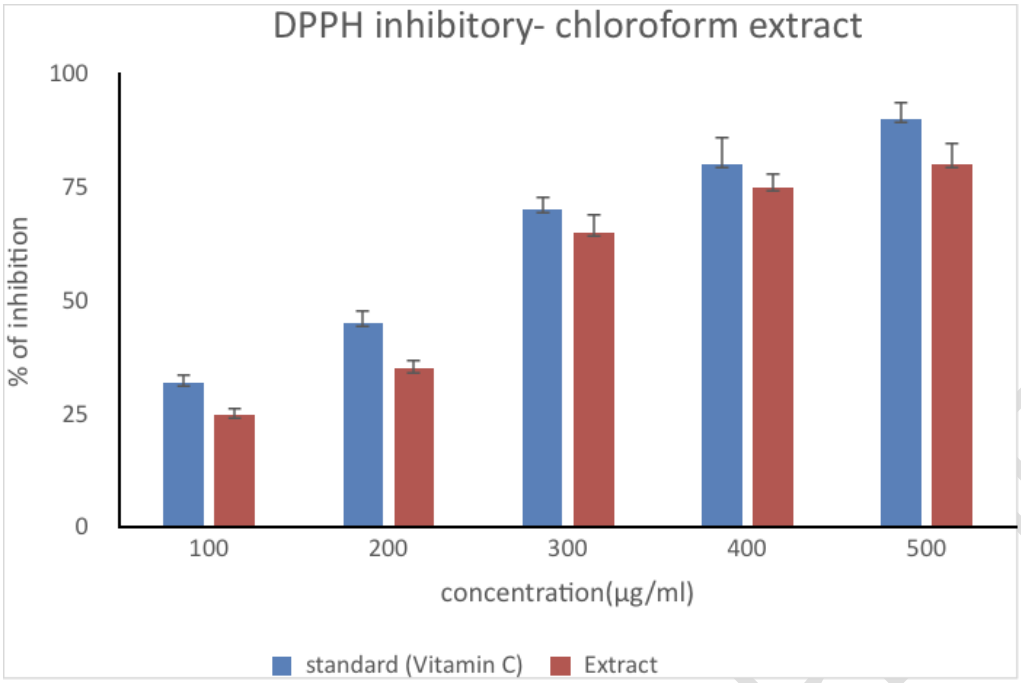

Figure 3: Represents bar graph showing DPPH inhibitory activity of chloroformic extractAndrographis echioides. Blue colour bar represents the percentage of inhibition of DPPH by the standard drug (vitamin C) and the red colour bar represents the percentage of inhibition of DPPH by chloroformic extract of Andrographis echioides. Each bar represents the mean ± SD of 5 observations. Significance at the levels of $P < 0.05$.a-compared with 100 µg; b-compared with 200 µg.

DISCUSSION

Free radicals are molecules, capable of independent existence and contain an unpaired electron in its outermost orbital. The presence of unpaired electrons makes it unstable and highly reactive. They either try to donate an electron or accept an electron to attain stability. Some examples of free radicals formed in the body include, hydroxyl radical, superoxide radical, anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical and peroxynitrite radical (19,20). These are highly reactive molecules which are formed in nucleus and membranes of the cell as byproducts of metabolism and cause damage to biologically relevant molecules like DNA, protein, carbohydrates and lipids(21,22).

Excessive generation of free radicals in the body leads to oxidative stress, which is a condition of oxidative damage that arises due to the imbalance between free radical production and antioxidant defense(23–25). Free radical mediated oxidative stress is considered as the major

cause for many diseases such as cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age-related macular degeneration(26)(27)(28). Antioxidants counteract oxidative stress by neutralising the free radicals, thereby it acts as a radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents (24,29). Previous studies have found that antioxidants help to reduce vision loss due to age-related macular degeneration in older people(30)(22)(31).

The sources of antioxidants include endogenous antioxidants present in the body and exogenous food sources like vegetables, fruits and other synthetic sources. Examples of antioxidants that come from outside the body include vitamin A, vitamin C, vitamin E, beta-carotene, lycopene, lutein, selenium, manganese, zeaxanthin. Flavonoids, flavones, catechins, polyphenol and phytoestrogen are all types of antioxidants mainly found in plant based foods(30)(32).

The present study focussed on the in vitro antioxidant property of *Andrographis echioides,* a medicinal plant widely used in the treatment of various diseases. Aqueous, ethanol and chloroform extracts of the plant were examined and found that all the three extracts possess significant antioxidant potential which increases with the increase in the concentration of the extract. Previous study conducted on ethanolic extracts of leaves of *Andrographis echioides* showed that it has potential DPPH free radical scavenging activity and nitric oxide scavenging activity. The phytochemical screening of dry powder of *Andrographis echioides* showed the presence of flavonoids, alkaloids, sugar, etc (33,34)(35). Similarly, another study on phytochemicals present in ethanolic extracts of *Andrographis echioides* showed the presence of various active phytochemicals like alkaloids, anthraquinones, coumarins, flavonoids, phenol and tannins. Chloroformic extracts contained maximum terpenoids (33). Earlier studies indicated that presence of flavonoids and phenolic compounds in herbal extracts prevents oxidative stress(36). Thus the above studies are in accordance with the present study and proves that *Andrographis echioides* has potential antioxidant properties.

The limitations of the current study was that, since the raw materials used for the study were natural products, they might not be found everywhere and also they may get damaged during its cultivation in the laboratory. The future scope of the study is that Andrographis echioides can be

used to prepare antioxidant drugs for the treatment of various diseases after further invivo studies.

CONCLUSION

Aqueous, ethanolic and chloroformic extracts of *Andrographis echioides* showed effective antioxidant potential and it could protect the biological system from oxidative stress including ageing, cancer, diabetes and cardiovascular diseases. Thus it can be used as a natural antioxidant for the treatment of various diseases in the human body.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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