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Original Article

PHYTOCHEMICAL SCREENING, *IN VITRO* ANTIOXIDANT AND ANTI PROTEASE ACTIVITIES OF METHANOLIC EXTRACT OF *NILGIRIANTHUS HEYNEANUS* NEES STEM

JENSON JACOB*

Department of Biochemistry, Pazhassiraja College, Pulpally, Wayanad, Kerala 673579
***Email: jensonjacobs@rediffmail.com**

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ABSTRACT

Objective: The aim of this study was focussed on phytochemical screening, *in vitro* antioxidant and antiprotease activities of methanolic extract of *Nilgirianthus heyneanus* stem.

Methods: The stem of the plant was washed thoroughly, shade dried and coarsely powdered. The powdered material of Niligirianthus heyneanus stem was extracted with methanol using soxhlet apparatus. Preliminary phytochemical screening for carbohydrates, proteins, alkaloids, phytosteroids, flavonoids, glycosides, polyphenolics, saponins, tannins was done by following standard procedure. *In vitro* antioxidant activities of methanolic extract were assessed using DPPH, ABTS and total antioxidant capacity. *In vitro* anti-protease activity of the plant was analysed using trypsin as an enzyme and BAEE (N-benzoyl-L-arginine ethyl ester) as a substrate.

Results: The results showed that phytochemicals such as carbohydrates, proteins and amino acids, flavonoids, glycosides, tannins and polyphenolics are present in the methanolic extract of *Niligirianthus heyneanus* stem. The *in vitro* antioxidant and antiprotease activities of *Niligirianthus heyneanus* stem clearly showed that the plant has antioxidant and antiprotease activity.

Conclusion: From this work, it can be concluded that Niligirianthus heyneanus stem has the potential to be a strong antioxidant and protease inhibitor.

Keywords: Oxidative stress, Flavonoids, Free radicals, Niligirianthus heyneanus

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INTRODUCTION

Herbal medicine has been used to treat human disorders for thousands of years because they have a vast and various assortment of phytoconstituents, which provide a physiological action on human body. Novel compound synthesis from the medicinal plants, which are of potential use in medicine and other useful applications. Herbal medicine and their active compounds have shown therapeutic potentials. The major antioxidant property of medicinal plants is due to their phytoconstituents such as flavones, isoflavones, flavonoids, coumarin lignans, catechins and isocatechins.

There is increasing evidence for the role of free radicals in causing several disorders such as cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases and ageing [1]. Reactive oxygen species can mediate inflammation and tissue injury and this can be managed by antioxidants. Antioxidants are natural or synthetic substances that have been linked in removing free radicals from the body. Several synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene are not safe due to their toxicity. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants.

Many plants of antioxidant activity have been reported to have anti-inflammatory property [2]. Also, many plant species have been found in the search for novel antioxidants. So considerable attention has been directed towards the identification of medicinal plants with antioxidant and anti-inflammatory ability. The plant selected for the study was *Niligirianthus heyneanus* Nees.

Niligirianthus heyneanus Nees is an aromatic shrub from the Acanthaceae family. It is used as folk medicine in different Ayurvedic medicinal preparations [3]. The roots of *Niligirianthus heyneanus* have been used as the plant source of the drug 'Sahachara' in Kerala [4]. 'Sahachara' is widely used against rheumatism and several neurological disorders. The water and ethanolic extracts of the stem showed anti-inflammatory, immunosuppressant and analgesic activities [5]. Hypoglycemic and hypolipidemic effects of *Niligirianthus heyneanus* have been reported in alloxan-activated diabetic rats. [6]. Based on the above ethnomedical importance the present work focused on the phytochemical analysis, antioxidant and antiprotease activities of methanolic extract from the stem of *Niligirianthus heyneanus.*

MATERIALS AND METHODS

Collection and extraction of stem material

Niligirianthus heyneanus stem was collected from the Swaminathan Research Foundation, Wayanad, Kerala, India. The stem of the *Niligirianthus heyneanus* was shade dried and then powdered. The powdered stem material was extracted with methanol using a Soxhlet extraction apparatus.

Phytochemical screening of methanolic extract of stem material

Phytochemical analysis was done to evaluate the presence of phytochemicals in the methanolic extract of *Niligirianthus heyneanus* stem [7, 8].

In vitro antioxidant activities of methanolic extract of stem

DPPH radical scavenging activity

1,1-Diphenyl-2-picryl hydrazyl (DPPH) is a free radical scavenging assay for measuring the antioxidant activity of medicinal plants. In this assay, the reaction mixture contains 2.8 ml 100µM DPPH in methanol and was added with 0.2 ml of methanolic extract of stem at different concentrations. The mixture was incubated for 30 min and absorbance was recorded at 517 nm. Ascorbic acid is taken as standard and methanol without sample along with DPPH was taken as control [9].

ABTS radical scavenging assay

This assay generates the oxidation of ABTS (2, 2'-azinobis [3-ethylbenzothiazoline-6-sulphonate]) to a nitrogen-centered radical cation, ABTS•. The stock solution contains 7.4 mmol ABTS added

with 2.6 mmol potassium persulfate. The working solution is made by adding these solutions in equal amounts and allowed to react for 12 h in dark conditions. The mixture was then diluted by adding 1 ml ABTS solution with 60 ml methanol to get an absorbance of 1.1±0.02 at 734 nm. 150 µl of different concentrations of extracts of the stem was allowed to react with 2850 µl of ABTS solution for 2 h. The absorbance was taken at 734 nm using a spectrophotometer. IC50 values were also calculated [10].

Total antioxidant capacity (Phospho molybdenum assay)

Total antioxidant capacity is one of the important methods for determining the antioxidant activity of medicinal plants. The principle behind assay is the reduction of Mo(VI) to Mo(V) by the root extract to form a green coloured phosphate Mo(V) complex at acidic pH. 0.3 ml methanolic stem extract was added with 3 ml reagent solution which contains 0.6 M Sulfuric acid, 28 mmol Sodium phosphate 4 mmol Ammonium molybdate. Incubate the mixture for 90 min at 95 °C and was cool. The optical density was taken at 695 nm using a spectrophotometer [11].

***In vitro* anti-protease activity**

This assay was carried out using the spectrophotometric assay by Sigma Aldrich with slight modifications. Trypsin, the protease is used to find the anti-protease activity of different solvent extracts of root. The assay was done based on the hydrolysis of the substrate BAEE (N-benzoyl-L-arginine ethyl ester) at the ester linkage, which leads to an increase of optical density at 253 nm. The mixture consisted of 200 µl trypsin, 200 µl tests and incubates for 10 min. The reaction is started by adding 3 ml substrate BAEE ((N-benzoyl-L-arginine ethyl ester) and absorbance was determined at 253 nm. Phenyl methyl sulphonyl fluoride (PMSF) is used as standard.

RESULTS AND DISCUSSION

The methanolic extract of *Niligirianthus heyneanus* was screened for the presence of phytochemical compounds. The qualitative analysis of methanolic extract showed the presence of carbohydrates, glycosides, flavonoids, alkaloids, tannins, polyphenolics, proteins and amino acids. The extract showed the absence of phytosteroids, saponins, fixed oils, gums and mucilages. These phytoconstituents are reported to therapeutic as well as biological properties [12, 13]. The high polar index of methanolic solvent can make it a high variety of phytoconstituents than any other solvents did [14].

Table 1: Phytochemical analysis of *Niligirianthus heyneanus* stem

Phytochemical constituent	Methanolic extract
Carbohydrates	+++
Proteins and amino acids	+++
Glycosides	+++
Alkaloids	+++
Phytosteroids	---
Flavanoids	+++
Saponins	___
Tannins and Polyphenolics	+++
Fixed oils and fats	___
Gums and mucilages	___

In vitro antioxidant activities of methanolic extract of stem

DPPH radical scavenging activity

DPPH assay is one of the procedures for screening the antioxidant capacity of herbal extracts. Here the percentage of scavenging of the methanolic extract is 67.15±1.87 at a maximum concentration of 1000 µg/ml. The IC50 value of methanolic extract was found to be 664.5 µg/ml. DPPH is a stable free radical with red colour having maximum absorption at 517 nm. These free radicals are scavenged by the antioxidants present in the stem sample of *Niligirianthus heyneanus*, which turned its colour to yellow [15]. Antioxidants with DPPH radical scavenging ability could donate hydrogen to free radicals, particularly to the lipid peroxide radicals that are the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation [15].

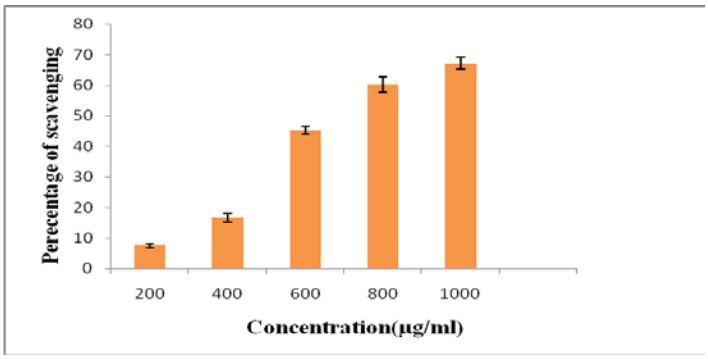

Fig. 1: *In vitro* free radical scavenging effect of the Methanolic extract by DPPH method

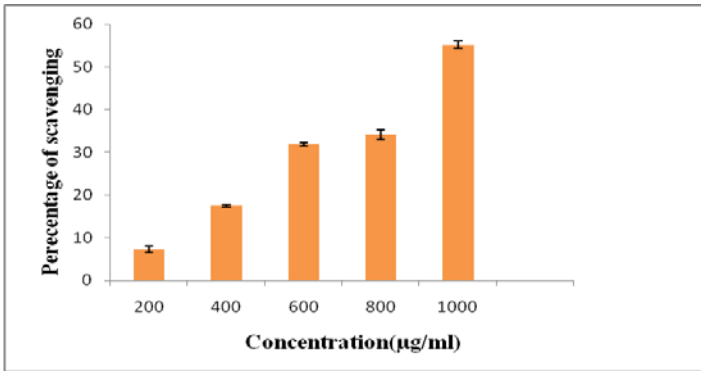

Fig. 2: *In vitro* free radical scavenging effect of the methanolic extract by ABTS assay

Total antioxidant capacity (Phospho molybdenum assay)

The total antioxidant capacity of plant extracts was determined by Phospho molybdenum assay. Here the methanolic extracts of *Niligirianthus heyneanus* stem showed increased total antioxidant capacity with increasing concentration. 1000µg/ml of the methanolic extract of *Niligirianthus heyneanus* stem was equivalent to 40µg/ml of the ascorbic acid standard. The assay leads to the transfer of Mo (VI) to Mo (V) by the methanolic extracts which have antioxidant activity resulting in green phosphate Mo (V). The electron-donating capacity of antioxidants depends upon its structure and series of redox reactions occurring in the activity [16].

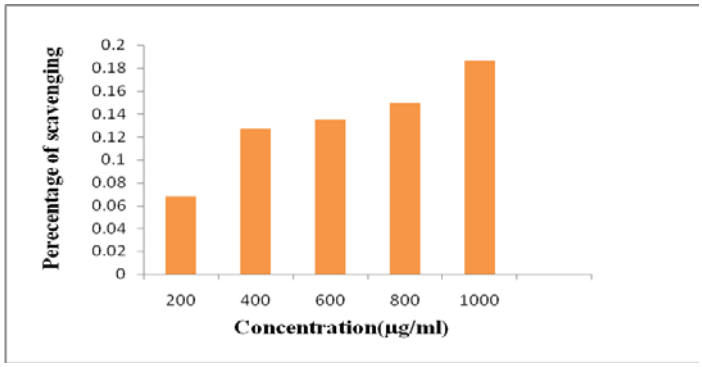

Fig. 3: Total antioxidant capacity of methanolic extract of *Niligirianthus heyneanus*

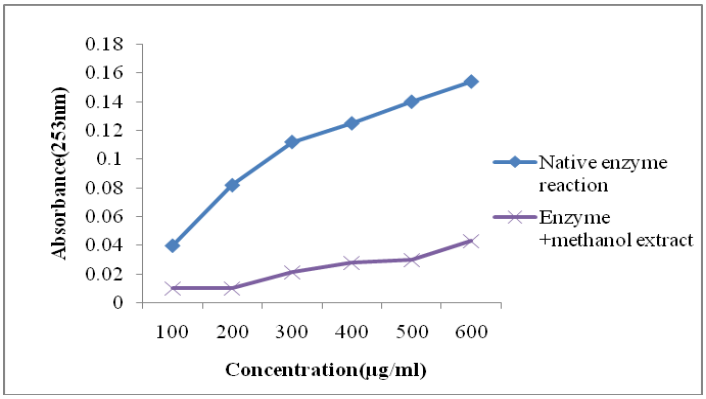

Fig. 5: *In vitro* anti-protease activity of *Niligirianthus heyneanus* stem

***In vitro* anti-protease activity**

Proteases are enzymes, which are involved to hydrolyze the peptide bond of protein. They play a major role in various biological actions at a physiological level as well as during an infection. In this study, the methanolic extract of *Niligirianthus heyneanus* stem showed 60.52 % inhibition at a suitable concentration of 500µg/ml. Protease inhibitors have medicinal properties in humans against inflammatory, immunological, respiratory disorders viral, parasitic infections and cancer. Here the methanolic extract of *Niligirianthus heyneanus* stem has better anti-protease activity.

CONCLUSION

In this study, *Niligirianthus heyneanus* Nees was tested to evaluate the presence of phytoconstituents, their antioxidant and antiprotease activities. The plant was collected and extracted with methanol using a Soxhlet apparatus. The evaluation of the methanolic extract of plant material showed the presence of several phytoconstituents. The antioxidant activity was measured by DPPH, ABTS and Total antioxidant capacity, which was proved to be better. The results showed that phytochemicals such as carbohydrates, proteins and amino acids, flavonoids, glycosides, tannins and polyphenolics are present in the methanolic extract of *Niligirianthus heyneanus*. The *in vitro* antioxidant and antiprotease activities of Niligirianthus heyneanus plant clearly showed that the plant has antioxidant as well as antiprotease activity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

The authors declare that there was no conflicting interest.

REFERENCES

1. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001;54:176-86.
2. Gupta SS. Prospects and perspectives of natural plants products in medicine. Indian J Pharmacol 1994;26:1-12.
3. Pullaiah T. Encyclopaedia of world medicinal plants. Vol. 4. Regency Publications, New Delhi; 2006.
4. Sivarajan VV, Balachamdran I. Ayurvedic drugs and their plant sources. Oxford and IBH publishing Co. Pvt. Ltd, New Delhi; 1994. p. 402-11.
5. Nair RB, Ravisankar B, Vijayan NP, Saraswathy VN, Sasikala CK. Anti-inflammatory effect of Niligirianthus heyneanus (Sahachara)-biochemical study. Bull Medico-Ethno-Bot Res 1985;6:196-206.
6. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, *et al*. Hypoglycemic and hypolipidemic effect of *Niligirianthus heyneanus* in alloxan induced diabetic rats. J Med Plants Res 2008;9:246-9.
7. Trease G, Evans SM. Pharmacognosy. 15th ed. London: Bailer Tindal; 2002. p. 23-67.

8. Harborne JB. Phytochemical methods-a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; 1984. p. 14-6.
9. Tirzitis P, Bartosz G. Determination of antiradical and antioxidant activity: basic principles and new insights. Acta Biochimica Polonica 2010;57:139-42.
10. Thaipong K, Boonprakob U, Crosby K. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Composition Anal 2006;19:669-75.
11. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269:337–41.
12. Benedec D, Vlase L, Oniga I, Mot AC, Damian G, Hanganu D, *et al.* Polyphenolic composition, antioxidant and antibacterial activities for two romanian subspecies of *Achillea distans* waldst. e1t Kit. ex Wild. Molecules 2013;18:8725–39.
13. Charalampos P, Konstantina L, Olga KM, Panagiotis Z, Vassileia JS. Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants 2013;2:11–22.
14. Paulsamy S, Jeeshna MV. Preliminary phytochemistry and antimicrobial studies of an endangered medicinal herb exacum bicolor Roxb. Res J Pharm Biol Chem Sci 2011;2:447–57.
15. Lu YR, Yeap Foo L. Antioxidant activities of polyphenols from sage (Salvia officinalis). Food Chem 2001;75:197–202.
16. Pellegrini N, Proteggente A, Pannala A, Yang M, Rice Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad Biol Med 1999;26:1231–7.
17. Gupta K, Maurya S, Agarwal S, Kushwaha A, Kumar R. Antioxidant assessment of extracts obtained through hot extraction process. Cell Mol Biol 2016;62:129.