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RESEARCH ARTICLE

Phytochemical identification of *Nilgirianthus ciliatus* by GC-MS analysis and its DNA protective effect in cultured lymphocytes

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ABSTRACT

Aim: To identify the phytochemical constituents and antioxidant activities of ethanol leaf extract of *Nilgirianthus ciliatus* (NC) and its protective effect against H_2O_2-induced DNA damages in cultured lymphocytes.

Materials and Methods: The fresh leaves of NC (1000g) were shade dried at room temperature for 30 days and the dried leaves was made into a fine powder. The ethanol leaf extract obtained was dried and used for phytochemical identification by GC-MS analysis, *in vitro* radical scavenging assays and DNA protection in lymphocytes.

Results and Discussions: The phytochemical screening studies identified sixteen chemical constituents present in the leaf extract of NC. The ethanol extract of NC (20, 40, 60, 80, 100 µg/ml) exhibited a significant dose dependent inhibition of *in vitro* radical scavenging assays and their corresponding IC_{50} values as follows such as Superoxide anion (32.00 ± 0.33 µg/ml) and DPPH radical (47.11 ± 0.50 µg/ml). The DNA protection was observed at 60 µg/ml of NC against 500 µM H_2O_2 treated lymphocytes.

Conclusion: Thus, our results show that NC exhibits *in vitro* radiacal scavenging assays and offers protection against H_2O_2-induced DNA damage in cultured lymphocytes and it can be developed as an effective antioxidant during oxidative stress.

Keywords: *Nilgiranthus ciliatus*, Antioxidants, Comet assay, Lymphocytes, Oxidative stress.

1. INTRODUCTION:

Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules in healthy human cells and cause protein and DNA damage along with lipid peroxidation.[1] Many phytochemicals have been found to play as potential antioxidants and antimicrobials. Antioxidants are provided to living organisms to protect them from damage caused by uncontrolled production of reactive oxygen species (ROS) and the concomitant lipid peroxidation, protein damage and DNA-strand breaks.[2] Current interest is focused on the potential role of antioxidants and antioxidant enzymes in the treatment and prevention of atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus and several others diseases.[3] *Nilgirianthus ciliatus* (Acanthaceae) has received considerable attentiondue to its wide range of secondary metabolites and its traditional usage in Indian system of medicine. It is a common herb, growing up to a height of 1.2 M and it is distributed in Western Ghats from South Kanara to Travancore.[4] The roots are bitter, sweet, thermogenic, emollient, diuretic, febrifuge, diaphoretic, depurative, anti-inflammatory, expectorant and tonic. Traditionally, the plant has been used for the treatment for rheumatalgia, lumbago, sciatica, limping, chest congestion, strangury fever, leucoderma, skin diseases, inflammation, cough, bronchitis, odontalgia and general debility. The leaves and bark are diaphoretic, expectorant, depurative and febrifuge, and are useful in whooping cough, fever, bronchitis, dropsy, leucoderma, leprosy, pruritus, inflammation, scrofula and fever.[5]

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Thus the aim of our present study is to investigate the phytochemical identification by GC-MS analysis, *in vitro* radical scavenging assays and to study the protective effect of NC against H_2O_2-induced DNA damage in cultured lymphocytes.

2. MATERIALS AND METHODS

2.1. Chemicals

2,2'azobis-3-ethylbenzthiazolie-6-sulfonic-acid (ABTS), 1,1-diphenyl-2-picryl hydrazil (DPPH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), 5′,5′-dithio(bis)-2-nitrobenzoic acid (DTNB), nicotin amide adenine dinucleotide (NAD), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), 5′,5′-dithio(bis)-2-nitrobenzoic acid (DTNB), Histopaque-1077, RPMI-1640 and nicotin amide adenine dinucleotide (NAD) and ascorbic acid were purchased from M/s. Sigma Chemical Co., St Louis, USA. All other chemicals and solvents were of analytical grade and obtained from Himedia, Mumbai, India.

2.2. Plant collection and preparation of the extract

Fresh leaves of *Nilgirianthus ciliatus* (NC) were collected from Trivandrum district, Kerala, India. The plant specimen was authenticated by Mrs. Padmaja, an expert in the field of Botany and the specimen was deposited in Ayurveda Research Institute for Mother and Child Health Care (ARIMCHC), Trivandrum. The fresh leaves of NC (1000 g) were shade dried at room temperature (28 ± 2ºC) for 30 days and the dried leaves was made into a fine powder (particle size-0.25 mm) by using an electric blender. About 250g of dried leaf powder were extracted with 750 ml ethanol (60–80ºC) for 2–3 h in a Soxhlet apparatus. The ethanol extract obtained was dried and used for *in vitro* radical scavenging, phytochemical identification and DNA protective effect in the cultured lymphocytes.

2.3. GC-MS analysis

GC-MS analysis was carried out at Indian Institute of Crop Processing Technology (IICPT), Thanjavur, India, GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1 fused silica capillary column (30mm x 0.25mm ID x1µmdf, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70ev; Helium (99.999%) was used as carrier gas at a constant flow of 1 ml/min and an injection volume of 2 µl was employed (Split ratio of 10:1); Injector temperature 250ºC; Ion-source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min) with an increase of 10°C/min, to 200°C, then 5ºC/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min.

2.4. *In vitro* radical scavenging assay

The antioxidant activity of the plant extract was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Bilios.[6] A measurement of superoxide anion scavenging activity of the extract was performed based on the method described by Nishimiki *et al.*[7] The percentage of inhibition was calculated by comparing the absorbance values of control and test samples. All the tests were performed for six times and the graph was plotted with the average of six observations.

2.5. Comet assay

Lymphocytes were isolated from the whole blood by the method of Boyum (1968) with slight modifications.[8] The cells were divided into four groups with the density of 1 x 10^6 cells in 2 ml culture media. Group-1 served as control that was treated with 0.05% DMSO. Group-2 cells were treated with 500 µM H_2O_2 as an oxidative stimulus for 5 min at 37°C. Group-3 cells were pretreated with 60 µg/ml (effective dose) of extract for half an hour prior to the treatment with 500 µm H_2O_2. Group-4 cells were treated with 60 µg/ml of extract alone for half an hour. After 5 min exposure of lymphocytes to H_2O_2, the cells were subjected to the comet assay by the method of Singh (2000).[9]

2.6. Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using statistical package of social science version 10.0 for windows. The values are expressed as mean ± SD for six experiments in each group and the P values <0.05 was considered as level of significance.

3. RESULTS

3.1. Phytochemical identification by GC-MS analysis

The GC-MS analysis of plant extract revealed the presence of sixteen chemical compounds (Phytochemical constituents) that could contribute the medicinal properties of the plant. The identification of the active principles present in the leaf extract was confirmed based on the peak area, retention time, molecular formula, molecular weight and peak area in percentage were shown in Table 1 and Figure 1.

3.2. *In vitro* radical scavenging assay

The results obtained by DPPH and Superoxide anion radical scavenging assay are shown in Figure 2. The ethanol extract of NC exhibited a significant dose dependent inhibition of DPPH activity, with a 50%. Inhibition (IC_{50}) at a concentration of 47.11 µg/ml as compared with the standard ascorbic acid (50.11 µg/ml). NC was found to possess good scavenging activity on superoxide radicals and at 100 µg/ml (p 0.05), it exerts optimum scavenging effects. Since the optimum dose was calculated as 60 µg/ml, further increase in the

concentration of NC beyond the optimum dose did not produce any significant increasing scavenging effect and this may be due to the saturation of the molecule in the system.

S.No	RT	Name of the compound	Molecular formula	MW	Peak area %
1	10.09	3-Octyne,2,2,7-trimethyl	$C_{11}H_{20}$	152	0.40
2	11.35	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	17.35
3	12.82	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	0.32
4	13.08	(R)-(-)-(Z)-14-Methyl-8-hexadecen-1-01	$C_{17}H_{34}O$	254	0.62
5	13.14	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.20
6	14.26	1-Dodecanol,3,7,11-trimethyl-	$C_{15}H_{32}O$	228	0.67
7	14.61	Phytol	$C_{20}H_{40}O$	296	23.59
8	15.03	2-n-Heptylcyclopentanone	$C_{12}H_{22}O$	182	0.37
9	15.36	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	0.70
10	16.96	2-Propenoic acid, 2-(dimethylamino)ethyl ester	$C_7H_{13}NO_2$	143	1.07
11	24.30	Squalene	$C_{30}H_{50}$	410	25.97
12	27.45	β-Tocopherol	$C_{28}H_{48}O_2$	416	0.90
13	28.54	Vitamin E	$C_{29}H_{50}O_2$	430	1.55
14	30.02	Campesterol	$C_{28}H_{48}O$	400	3.60
15	30.49	Stigmasterol	$C_{29}H_{48}O$	412	7.72
16	32.36	β-Amyrin	$C_{30}H_{50}O$	426	14.95

Table 1 Phytocomponents identified in the ethanol leaf extract of *Nilgirianthus ciliatus* by GC-MS analysis

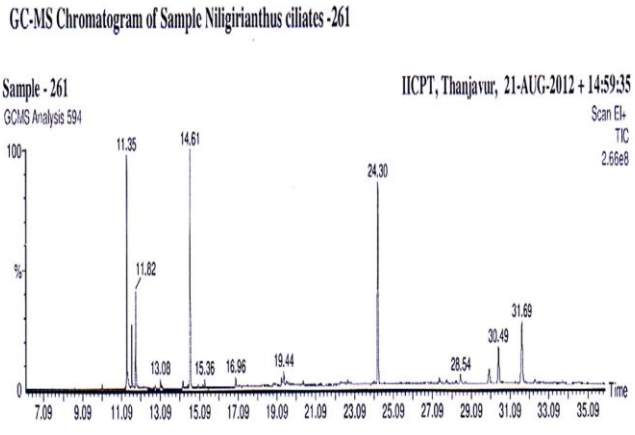

Figure-1

Figure 1 GC-MS analysis of ethanol leaf extract of *Nilgirianthus ciliatus*

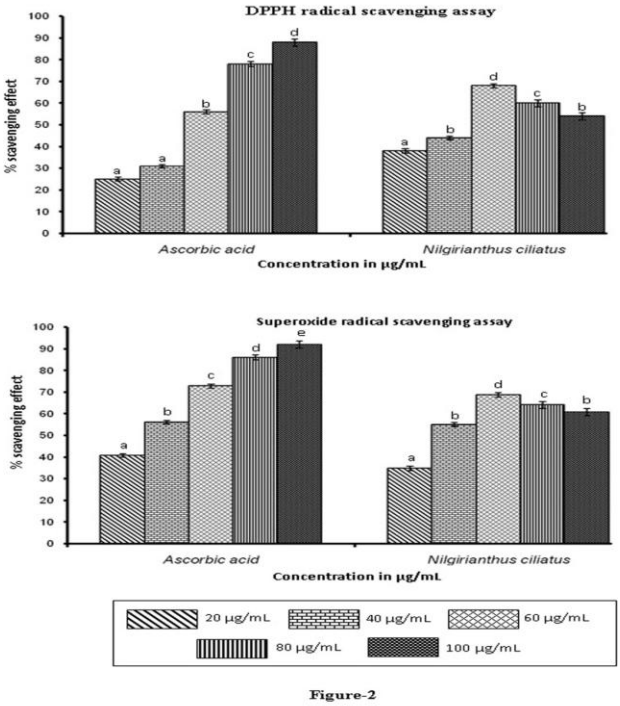

Figure-2

3. Values are given as mean ± SD of six experiments in each group. Bar values are sharing a common superscript (a,b,c,d) differ significantly at P <0.05 Duncan's Multiple Range Test (DMRT).

Figure 2 DPPH and Superoxide anion radical scavenging effect of *Nilgirianthus ciliatus* with different concentrations in comparison with standard ascorbic acid.

3. Comet assay

Figure 3 shows the changes in the protection of cultured lymphocytes from H_2O_2 induced DNA damage when treated with 60 µg/ml of NC. There was a significant increase in the levels of DNA damage (% DNA in tail, tail length, tail moment and olive tail moment) at a dose of 500 µM of H_2O_2 treated lymphocytes. The extent of DNA damage was reduced in a concentration of 60 µg/mL of NC pretreated lymphocytes when compared with H_2O_2 treated group. NC alone treated control group did not show any significant changes in comet tail formation when compared to normal lymphocytes.

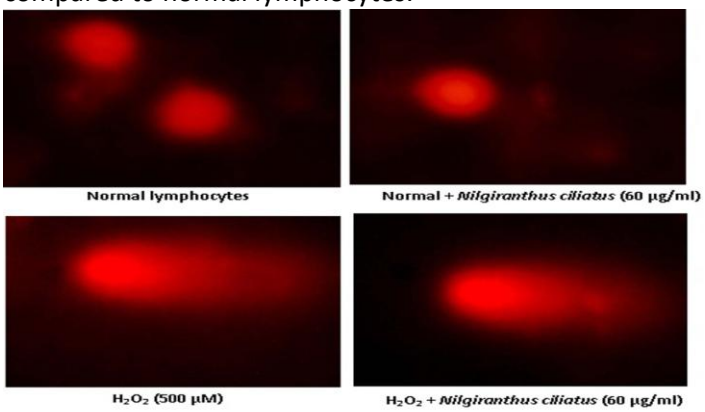

Figure-3

Figure 3 Changes in the levels of Comet assay (% DNA in tail, Tail length, Tail moment and Olive tail moment) in cultured lymphocytes on pretreatment with ethanol extract of *Nilgirianthus ciliatus* (NC).

4. DISCUSSION

Phytochemicals are natural and non-nutritive bioactive compounds produced by plants that act as protective agents against external stress and pathogenic attack.[18] Secondary metabolite is a crucial for plant defenses (e.g. as an antioxidant or antimicrobial agent) which has enabled plants to survive. Based on their biosynthetic origin, phytochemicals can be divided into several categories: phenolics, alkaloids, steroids, terpenes, saponins, etc. Phytochemicals could also exhibit other bioactivities such as antimutagenic, anticarcinogenic, antioxidant, antimicrobial, and anti-inflammatory properties.[11] These plant-derived phytochemicals with therapeutic properties could be used as single therapeutic agent or as combined formulations in drug development.[12] The present study is to investigate the phytochemical identification of ethanol extract of NC by GC-MS analysis, antioxidant ability and DNA protection by performing various *in vitro* assays and the results indicated a concentrated dependent antioxidant ability of NC. The phytochemical screening studies have been carried out by GC-MS analysis and we identified the sixteen chemical constituents present in the leaf extracts of NC. The results of our studies indicated that 60 µg/ml concentration of NC showed optimum protection against free radical induced oxidative damage. The DPPH and superoxide radical scavenging activity of NC can be attributed to the presence of phytol, squalene, β-tocopherol, vitamin E, campesterol, stigmasterol and β-amyrin which donates hydrogen and an electron to hydroxyl radicals, stabilizing them and giving rise to a relatively stable radical. Thus, the free hydroxyl group on the aromatic ring is responsible for the antioxidant properties.

The protective effect of DNA which might be due to the presence of vitamin E, campesterol, β-amyrin, stigmasterol, squalene, β-tocopherol and phytol present in the ethanol leaf extract of NC inhibit the oxidative stress induced DNA damage in cultured human lymphocytes.

5. CONCLUSION

Thus, from the results obtained we observed that NC showed significant antioxidant potential in terms of scavenging free radicals produced by various *in vitro* assays and it also protects from H_2O_2-induced DNA strand break formation in cultured lymphocytes.

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Conflict of Interest: None Declared

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