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Amaranthus Viridis DNA Polymerase, Antioxidant, Antimicrobial and Colon Cancer Study

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Abstract: *Pathogenic and non-pathogenic infection is one of the major burden on society form past and future days. It could be reduced by providing g proper diet and medicine and also reduce the risk of diseases. Our present work had carried out on methanol extracts of green and pink Amaranthus Viridis to investigate some medicinal properties. Amaranthus Viridis are analyzed for their polymerase inhibition, antioxidant, antimicrobial and colon cancer cell proliferation activities. The present results suggest that all the assayed Amaranthus Viridis leaves are having DPPH antioxidant potential A1 had the highest 66.59 % antioxidant properties. Methanol extract of aerial parts of Amaranthus Viridis was checked for antimicrobial activity against various pathogens like Micrococcus luteus indicated that the A1was the most potent activity (78.96% inhibition) as compared to the other extracts. B1 shows maximum polymerase activity compare to other extracts. Colon cancer cell proliferation study suggests A1 to B2 activity was decreased cell line inhibition activity.*

Keywords : Amaranthus Viridis, DNA polymerase, Antioxidant activity, antimicrobial activity, colon cancer

INTRODUCTION

Day by day changes in the modern living life style, works, diets and habitats are may be responsible for the generation of various noninfectious diseases like cancer CVD etc or may change the normal balance of health. Such diseases can be decreases or control by diets canting vegetables and fruits. Epidemiological report suggest that the vegetables, grain cereals and fruits are having beneficial role against reduction in risk of infectious and noninfectious diseases like cancer, neuro-degenerative diseases ,CVD, HIV gout, diabetic etc. The protective effects of vegetables and fruit may be contributed by their secondary metabolites such as ascorbic acid, carotenoids and polyphenols like flavonoids anthocynin Catechin etc called as Phytochemicals (Funde, 2011, 2012). These phytochemicals may help to protect cell from relieve oxidative stress,(i.e. preventing free radicals from damaging macromolecules such as proteins, DNA and lipids or inhibit the various biochemical pathways) (Funde, 2012). Generally free radicals are responsible for causing various diseases like cancer CVD etc. Such radicals can be scavenging by certain phytochemicals present in fruit and vegetables. But still in all over world so many people are not eating the vegetables and fruits may be because of taste flavor etc. Interest of people towards vegetable and fruits can be generated by giving scientific evidences regarding fruits and vegetables (Funde, 2012). This can be translated into culturally appropriate health messages for promoting increased consumption of these vegetables. The work would be helpful for improving nutritional status and reducing the risk of diet-related diseases among

community. *Amaranthus Viridis* (Tanduliya) vegetables is widely consumed in India and many countries. It has wide application in traditional medicinal system. Previous study found that antioxidant, antimicrobial, Anthelmintic, Antipyretic, Anti-inflammatory, Antidiabetic and antihyperlipidaemic activities. (Kumar et al., 2009, 2010a, 2010b, 2011; Iqbal et al., 2012; Carminate et al., 2012; Sravan et al., 2011; Pandhare et al., 2012; Girija et al., 2011)

In the present study was carried out on analysis total polymerase inhibition activity, antioxidant, antimicrobial and anticancer activities of green leafy vegetables from India.

Material Method

Chemicals and Instruments

AccuTaqg polymeras, Template DNA, dNTPs, forwarding primer, reverse primer, agarose, Tris-acetate-ethylenediaminetetraacetic acid, DMSO, PBS, Dulbeccos Modified Egal Medium, fetal bovine serum media, Luria Britannia broth, (Himedia), MTT, DPPH and methanol (HPLC gradient grade) Merck (Germany). Microbial strain *Ecoil (ATCC- 25922), Styphylococcus epidermidis (ATCC- 12228)* and *Micrococus luteus (ATCC-9341)* was purchased from national chemical laboratory Pune, India. HT-20 cell line was taken from NCCS, Pune used for study of cell proliferation.

Extraction

Amaranthus Viridis (green color [older leaves (A1), younger leaves (A2)] and pink color [older leaves (B1), younger leaves (B2)]) plant materials were collected from Maharashtra (India) area. A plant was dried at 37^0C temperature and thereafter stored plastic bags. Different part of plant powder (1 gm) was mixed with 15 mL of methanol over a period of 24 hr. at 37 ^{0}C at shaking condition. Then mixture was filtered through Whatman filter paper final volume were made 15 mL.

Antioxidant activities by DPPH radical assay

Antioxidant activity was studied by using DPPH radical assay (Funde, 2015; Shaikh et al., 2019). 20 ul extracts in 100 ul MeOH and 100 ul of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in MeOH, and final volume was adjusted to 100 ul. Mixtures were vigorously shaken and left for 30 min in the dark. Absorbance was measured at 517 nm using MeOH as blank. 100 ul MeOH and 100 ul of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) in MeOH was used as control. IC_{50} of sample for neutralization of DPPH radical was calculated using the equation:

$$\%Inhibition = \frac{A - B}{A} \times 100$$

A= Absorbance of control, B = Absorbance of Sample

Polymerase inhibition assay

Polymerase inhibition assay was carried out by using Polymerase inhibition assay (Funde, 2015) Polymerase inhibition assay were contains final reaction volume 25.2 µL In brief reaction contains addition of 17.5 µL of distilled water 2.5 µL of AccuTaqg polymerase buffer 0.5 µL of Template DNA 2.5 µL dNTPs 1 µL of forwarding primer, 1 µL of reverse primer and 0.2 µL AccuTag polymerase such reaction is subjected for PCR. The PCR program is set as above Intial denaturation at 95 ◦C for 5 min. 35Cycle (such as denaturation at 95 sec annealing 62◦C fro 45 sec extension at 72◦C for 1 min) final extension at 72◦C for 5 min and hold at 20◦C for ∞. How ever methanol is used as positive controls. After PCR reaction A 15 µL aliquot of the mixture from reaction was loaded into 1.0% agarose gel containing ethidium bromide (0.05µg/mL) in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) buffer. The electrophoresis was carried out for 30 min at 100 V, and

then gels were illuminated with UV light and photographed. Sample used for study was 1:100 dilution of original extracts.

Antimicrobial activity

The antimicrobial testing of the plant extracts were determined using turbidometrically method as described (Funde, 2015; Shaikh et al., 2019). Extraction were carried out in different solvent system and solvent was evaporated on by using rotary evaporator till dry extract. Dried extract were dissolved in DMSO and used for study. Luria britania broth was used for sub-culturing the bacterial isolates. The organisms used were of the ATCC and NCIB. Bacteria were grown overnight at 37 ^{0}C in Luria britania broth under aerobic conditions for 18 hr. The inoculums suspension were standardized and then tested against the effect of the crude extracts. Test sample contains 2µL crude extract, 200 µL fresh medium and culture were added in 96 well plate. While control have 2 µL of 0.1% DMSO instead of extracts and remaining addition as like to test sample. Plates are incubated at 37 ^{0}C for 24 hours. The absorbance of plate were read at 600nm after 24 Hr. % inhibition calculated by following formulae

$$\%Inhibition = \frac{A - B}{A} \times 100$$

A= Absorbance of control, B = Absorbance of Sample

Anticancer assay

MTT or viable cell proliferation assay widely used for determination of anticancer activity of drugs and extract (Funde, 2015). In which NAD (p)H oxidoreductase enzymes of cell reducess the the MTT (3-(4,5 – dimethylthiazol – 2-yl) – 2, 5- diphenyltetrazolium bromide) purple dyes. Human breast carcinoma 1.25 X10^5 cells (HT20) per well was plated in 0.2 mL high glucose DMEM (Dulbeccos Modified Egal Medium) supplimented with 10% fetal bovine serum media. Plate were incubated for overnight in 5% CO_2 incubator to adhere cells. 50 µg of extracts add in well triplicate manner and control contains only and media. Plate was incubated in 5% CO_2 incubator for 24 hr. MTT assay was carried out to measure viable cells in details, Media was removed from plates carefully and cells washed with phosphate buffer. 100 µL (5mg/mL MTT in PBS) of MTT was added and plate incubated in 5% CO_2 incubator for 4 hr. Assay plate was kept in dark for incubation. After incubation MTT removed carefully and 100 µL DMSO was added presence of viable HT20 cell visualised by purple color. Absorbance of plate measured at 595nm in presences of DMSO as blank. Calculation was carried out by using following formula-

$$\%Inhibition = \frac{A - B}{A} \times 100$$

A= Absorbance of control, B = Absorbance of Sample

Result and Discussions

Antioxidant activity

Table 1: Antioxidant activity of *Amaranthus Viridis* extracts.

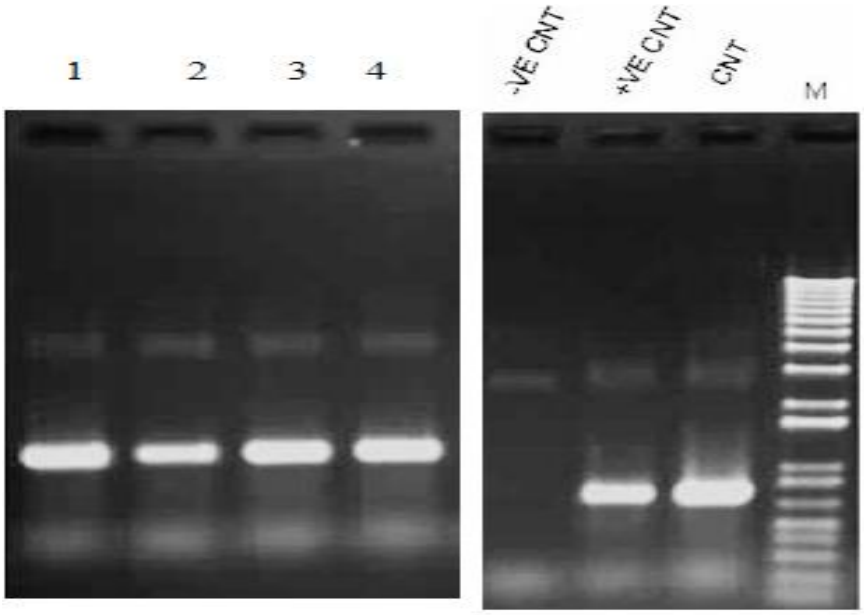
Sr. No.	% Inhibition DPPH radicals
A1	66.59 ± 0.87
B1	45.93 ± 0.51
A1	10.62 ± 0.60

B1	9.87 ± 0.24

The free radical scavenging effects of *Amaranthus Viridis* was tested using methanolic solution of the DPPH free radical which exhibits a deep purple colour with maximum absorption at 517 nm. The methanol extracts studied exhibited significant activity towards scavenging DPPH radicals, ranging from 9.87 to 66.59 % inhibitions (Table 1). Older leaves of *Amaranthus Viridis* had maximum antioxidant as compare to younger leaves. Presence of antioxidant activity in vegetables could be able to fight against the various diseases (Funde, 2011).

Polymerase inhibition assay

Several types of organism are contains DNA polymerase enzyme which catalyses the addition of deoxyriboneucleotides to 3 hydroxy terminus of primer double strand DNA molecules. Polymerases are essential for DNA replications, repair and recombination. It is also have major role in cell division process. Previous research works suggest that higher polymerase activity observed in pathogenic infected cell and in caner cell as compare to normal cells. In such case find out polymerase inhibitors is very important (Funde, 2015; Funde, 2015). So that our present study was carried out on PCR based polymerase inhibitory action of *Amaranthus Viridis* leaves extracts. Result obtained from study was mentioned fig. 1 *Amaranthus Viridis* extracts are not able to inhibit the polymerase enzymes. Well 2 indicates the partial inhibition of polymerase enzyme where as other extracts of *Amaranthus Viridis* are not able to inhibit the enzyme. The inhibition is depends on the concentration of the inhibitor present in the extracts.



PCR Image - well 1 - B1, well 2 – A1, well 3 – B2, well 4 - A2 , -VE CNT – negative control, +VE CNT – positive control, CNT – control and M – marker DNA

Antimicrobial activity

Table 2: Antimicrobial activity of *Amaranthus Viridis* extracts.

Plant name	% inhibition *Basillus subtilus*	% inhibition *Styphylococcus epidermidis*	% inhibition *Micrococus luteus*	% inhibition *Ecoil*
A1	25.12 ± 0.53	59.61±0.19	78.96 ±0.61	1.51 ±0.26

A2	10.75 ±0.81	5.12 ±0.57	48.96 ±0.74	13.78 ±0.48
B1	19.8 ±0.70	23.39 ±0.68	54.13 ±0.30	13.44 ±0.9
B2	0.73 ±0.14	2.88 ±0.07	66.55 ±0.89	7.23 ±0.72

Different medicinal plants and *Amaranthus Viridis* extract had shown antimicrobial activity against gram positive and gram negative bacteria. Current study of antimicrobial activities of *Amaranthus Viridis* leaves were tested by using methanol extracts. The concentration of *Amaranthus Viridis* shows inhibitory effects on the tested microorganisms. The results of the test of microorganisms are given in Table 3. Strains like *Styphylococcus epidermidis* and *Micrococus luteus* higher antimicrobial activity as compaire to *Ecoli.* In general older leaves of *Amaranthus Viridis* methanol extracts exhibited very high antimicrobial activities against *Micrococus luteus* compared to the younger leaves of *Amaranthus Viridis*. The results of the antimicrobial screening for Ecoli showed low activity against both the extract of *Amaranthus Viridis*. Antimicrobial activities is observed due to many natural product have been found to be responsible for several biological properties, including antimicrobial properties (Funde, 2012; Iqbal et al., 2012; Carminate et al., 2012).

Anticancer assay

Table 3: Anticancer activity of *Amaranthus Viridis* extracts.

Sr. No.	HT-29 IC_{50}
A1	9.92 ±0.38
A2	1.75 ±0.25
B1	5.1 ±0.64
B2	ND

Generally presence of natural products was found to increase the anticancer activity. Variation in activity may be varying from plant to plant or different part of the same plants because of its phytochemicals concentration. This is in contrast to the results obtained for *Amaranthus Viridis* leaves obtained here because of its active ingredients. A1 and B1 extracts contain maximum activity as compare to other extracts. The increase in anticancer activity in A1 might be because of higher antioxidant and polymerase inhibition activities of A1 extracts.

Conclusion-

Green vegetables are widely consumed in world wide has proven the medicinal importance of green vegetables. *Amaranthus Viridis* are having different activity like antioxidant, antimicrobial and anticancer activities. Overall, findings of this study support that *Amaranthus Viridis* aerial parts can be utilized as an effective and safe source of functional food materials of natural antioxidants.

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