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Anti-proliferative effect of *Ceriopsdecandra* derived fraction against Human gastric cancer on AGS cell line

Thirunavukkarasu Palaniyandi.[1,], Rohith Kumar Reddy [1] Sudhakar Natrajan[1]

Mohan Ranganathan[1,] Rajeswari Hari[1], Asha Sivaji[2], Sandhiya Viswanathan[1]

[1]Dr.M.G.R. Educational and Research institute, Maduraivoyal, Chennai-95

[2]Department of Biochemistry, D.K.M College for Women, Vellore, Tamil Nadu

India.

Corresponding Author

Dr. P. Thirunavukkarasu

Assistant Professor, Department of Biotechnology,

Dr.M.G.R. Educational and Research institute,

EVR Road NH4, Maduraivoyal,Chennai-95. Tamilnadu, India.

email. ppthirunacas@gmail.com,

Mobile: 91+9952172249

http://orcid.org/0000-0001-9216-419X

ABSTRACT

Mangrove plant species *C,decandra* have wide range of pharmacologic actions. Such as anti microbial, Antioxidant, anti-inflammatory and anti oralcancer activity. The present study aimed to evaluate the antiproliferative and biochemical properties of mangrove derived on human gastric cancer on AGS cancer.Theanti proliferative effect of *C.decandra* isolated

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bioactive compound berberine was determined by MTT assay, and the oxidative stress was determined by lipid peroxidation method along with assessment of changes in the enzymatic and non enzymatic antioxidant status. We observed intracellular reactive oxygen species(ROS) level by DCFH-DA method, mitochondrial membrane potential alteration by rhodamine 123 staining and apoptotic morphological changes by acridine orang/ethidium bromine dual staining method and Hoest staining assay and comet assay. Mangrove derived berberine enhances lipid peroxidative markers such thiobarbituric acid reactive substance conjugated diene, and lipid hydroperoxide in AGS cell lines. Mangrove derived fraction enhances the ROS levels, which is evidenced by the increased 2-7 diacetyl dichlorofluorescein fluorescence. Further, *C.decandra*treatment altered the mitochondrial membrane potential in AGS cells. Additionally, we observed increased apoptotic morphological changes in berberine treated group.Mangrove fraction exhibit potent antiproliferative effect on AGS cell line and upon further will going to check *invivo* experimental animal studies.

Keywords: Gastric cancer, *Ceriopsdecandra*, Apoptosis, Lipidperoxidation. Apoptosis, Antiproliferative.

Introduction

Cancer is a common term for a large group of disease which are characterized by uncontrolled cell growth and formation of tumors." Cancer cells can spread to any part of the body and affecting individuals from different sexes, ages and races(American Cancer Society, 2020). All cancers are multi factorial they include genetic, hormonal, physical, chemical, and environmental factors, metabolic disorders, infectious organism and an improper diet[2]. The treatment of cancer will depend on the type of cancer which involves a combination of treatments such as surgery, chemotherapy, radiation therapy

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and immunotherapy (Rodriguez*et al.*, 2018). Cancer is the most serious problem worldwide and is the second most common cause of death in the United States (Siegel *et al.*, 2020).

Though there are a number of plants available with anti-cancerous properties, still continuous effort has been given for a search of drugs of new plant origin other than common traditional plants sources. Mangrove have been known since the folk era to be highly useful and active against various diseases and are potent sources of bio active compounds including antioxidant, anti-diarrheal, anti-inflammation, anti-diabetic and also anticancer compounds(Bandaranayake,2002). Numerous bio active compounds of pharmaceutical important have also been reported from the mangroves ecosystems(Patra and Thatoi2011)

Gastric cancer arises from the gastric mucosa. The highest incidence of gastric cancer has been reported in men, older adults (60-84 years), and in eastern Asia, Eastern Europe, and South America (Thirumurthy*et al.*, 2013). Indeed, studies have shown that 52% of patients with gastric cancer are in Asia, and 41% are in China (Sasako*et al.*, 2010). The incidence of gastric cancer is 8.7 per 100,000 white men, compared with 17.2 per 100,000 Asian/Islander men(Kohler *et al.*, 2011). Surgery is considered as the primary method for the treatment of gastric cancer and to be the only strategy with curative possibilities. In the present study anti gastric cancer and anti-proliferative effect of mangrove plant species of *C.decandra* extract were studied.

Materials and Methods

Collection and Identification of Plant Materials

Leaves of *C.decandra* were collected from Pichavaram, South East coast of India, Tamil Nadu and authenticated in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, India. The leaves were carefully examined.

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Old, insect damaged and fungus infected leaves were removed. Healthy leaves were washed and spread out. They were shade dried, coarsely powdered and stored in air tight bottles for further work. The was carried out Department of biotechnology, Dr.MGR educational and Research institute, Maduravoyal, Chenna, from march 2018 to December 2018.

Extraction

The dried leaves powder was extracted by maceration with 80% EtOH. After filtration and evaporation of solvent under reduced pressure, the ethanol extract was fractionated. Fractionation of the ethanolic extract (10g) was carried out by suspending the extract in 100mL water and partitioned successfully with different organic solvents (chloroform, CH2Cl2 and n-BuOH) of increasing polarity by using separating funnel. The n-butanol fractions were dried by evaporating in a rotary evaporator and the crude extract was stored at 4°C till further analysis.

Chemicals

Thiobarbituric acid (TBA), Phenazine methosulphate (PMS), Nitro blue tetrazolium (NBT), 5, 5-dithiobis 2-nitrobenzoic acid (DTNB), 3-(4, 5-dimethyl-2-thiaozolyl)-2, 5-diphenyl-tetrazolium bromide (MTT), 2-7-diacetyl dichlorofluorescein (DCFH-DH), Rhodamine 123 (Rh 123), Ethidium bromide, Acridine orange, Cell culture chemicals such as heat inactivated fetal calf serum (FBS), Minimum essential medium (MEM), Glutamine, Penicillin-streptomycin, EDTA, Trypsin, Low melting agarose, Normal melting agarose, Phosphate buffered saline (PBS) and reduced glutathione (GSH) were purchased from Sigma chemical Co., St. Louis, USA.

Cell culture and maintenance

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The AGS gastric cancer cell line was maintained in Dulbecco's Modified Eagles Medium (DMEM) respectively and supplementation of 2mM l-glutamine and Balanced Salt Solution (BSS) adjusted to contain 1.5 g/L Na2CO3, 0.1 mM non-essential amino acids, 1mM sodium pyruvate,2mM l-glutamine, 1.5 g/L glucose, 10 mM HEPES and 10% fetal bovine serum (GIBCO, USA). Antibiotics of Penicillin and streptomycin (100 IU/100 g) were added to the medium and it's adjusted to 1mL/L. The cells were maintained at 37◦C with 5% CO2 in a humidified CO2 incubator for 48h.

Cell viability assay

Cell viability was analyzed by 3-(4,5-dimethylthiazol-2-yl)- 2,5-di-phenyl-tetrazolium bromide (MTT) assay, as described by Mosman 1983) Briefly, exponentially growing cells(1 × 105 cells/mL) were seeded in 96-well platesand were treated with different concentrations of isolated squalane in the series 5,10, 25 50, 100, 150,200, 250, 300, 350 and 400 µg/mL for 24 and 48h with FCS free complete medium. 100 L of MTT (5 mg/mL) was added to 24 and 48 htreated wells. After the plates were incubated at 37◦C for 4h, the supernatant was aspirated, and 200 L of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formosan crystals.Absorbance was measured at 620 nm using a 96-well microplate reader (THERMO Multiskan, USA).

Measurement of intracellular ROS in cells by spectrofluorimetric and fluorescence microscopic methods

Intracellular ROS was measured by using a nonfluorescent probe, DCFH-DA, which penetrates into the intracellular matrix of cells to be oxidized by ROS to fluorescent dichlorofluorescein (DCF) [10]. Cells were incubated for 24 h with different

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concentrations *C.decandra*extract. Fluorescent dye DCFH-DA was then added to the cells and incubated for 30 min The cells were washed with PBS to remove the excess dye before fluorescent measurements that were carried out with excitation and emission filters set at 485 ± 10 and 530 ± 12.5 nm, respectively (Shimadzu RF-5301 PC Spectrofluorometer, Japan). Fluorescence microscopic images were taken using the blue filter (450–490 nm) (Nikon, Eclipse TS100, Japan).(Rastog*et al.,* 2010).

Direct fluorescence microscopic analysis for apoptosis induction by AO/EtBr

1 L of a dye mixture (100 mg/mL acridine orange (AO) and 100 mg/mL ethidium bromide (EtBr), in distilled water) was directly stained with berberine treated cells grown on clean microscope cover slips. After staining the cancer cells were washed with PBS (pH 7.2) and incubated for 1 min, the cells were then visualized under fluorescence microscope (Nikon Eclipse, Inc., Japan) at 400× magnification with an excitation filter at 480 nm.

Analysis of mitochondrial membrane potential (Δψm) by Rhodamine 123 staining

The AGS Gastric cancer cells was seeded in 6 well plates (1 × 105 cells/well) and allowed to grow for a day before exposed to IC_{50} concentrations 150, 250, and 300 μg/mL isolated berberine. After the specific time intervals (24, 48 and 72 h), the cells were fixed in 4% paraformaldehyde, washed twice with PBS, and exposed to the Δψm specific stain Rhodamine 123 (Rh-123) (10 g/mL) for 30 min at 37◦C. The cells were then washed twice with methanol to remove the excess stain, washed again with PBS, and analyzed for changes in Δψm using fluorescence microscope with an excitation and emission wavelengths of 505 nm and 534 nm, respectively.

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Measurement of DNA damage by DNA fragmentation

DNA damages by apoptosis were evaluated by genomic DNA fragmentation. The cells (1 × 106 cells) were separately suspended in 10 mL of buffer containing 10 mM Tris–HCl and 10 mM EDTA(pH 8.0). The cells were then treated with 150,250 and 350 µg/mL isolated berberine in 10 mL of a solution containing10 mM Tris–HCl, 10 mM EDTA (pH 8.0) and 20 mg/mL proteinase K. The mixture was incubated at 37◦C for 3h, followed by DNA extraction with phenol:chloroform:isoamyl alcohol solution (25:24:1). The extracted DNA was treated with DNase free RNase at a concentration of 20 mg/mL at 4◦C for 45min and precipitated with 100 mL of 2.5 M sodium acetate and 3 volumes of ethanol. The DNA fragmentationanalysis was then carried out by electrophoresis using 10 g of the extracted DNA from the selected cancer cells for a period of 45 minat 100 V on a 2% agarose gel containing ethidium bromide and visualized under GelDoc System (Alpha Innotech Image Viewer, version6.0.0, Japan).

Estimation of reduced glutathione levels

The levels of reduced glutathione (GSH) were determined in the supernatant obtained after centrifuging the trypsinized cells according to the procedures described elsewhere(Kakkar*et al.*, 1984).

Estimation of antioxidant enzyme activity

The supernatant obtained after centrifuging the trypsinized cells was used for the measurement of activities of antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), according to the procedures described elsewhere (Ellman,1959)

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Estimation of lipid peroxidation markers

The cells were harvested by trypsinization. The pellet obtained was suspended in PBS and taken for the measurement of lipid peroxidative markers such as thiobarbituric acid reactive substances (TBARS), conjugated dines (CD), and lipid hydro peroxide (LHP), according to the procedures described elsewhere (Gurib-Fakim, 2006).

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 (SPSS) version 12.0 for Windows. The values were expressed as mean ± SD for six samples in each group. In addition, *p* value of < 0.05 was considered as statistically significant.

Results

MTT-Assay

The Effect of *C.decandra* on cell proliferation was determined by MTT assay. The proliferation of AGS cells was significantly inhibited by *C.decandra*. Fig. 1 (a-b)shows the changes in the percentage of cell death in control and *C.decandra* -treated cells. The treatment with 5,10,50 and 100µg/mL of *C.decandra* did not show significant ($p<0.05$) proliferation inhibition. The treatment with 65, 125 250 and 500 µg/mL of *C.decandra* significantly inhibited in AGS cells. Concentrations of 250 and 500 µg/mL displayed almost the same inhibitory effect (83and 89 % inhibition) on AGS cells. Exhibited a dose-dependent cytotoxicity against human Gastric cancer cell (AGS) and the inhibitory concentration (IC50) were found to be 288µg/mL at 24 h incubation respectively(Fig 1&2). In this study observed intracellular ROS levels by DCFH-DA method, mitochondrial membrane potential alterations by Rh-123 staining, oxidative

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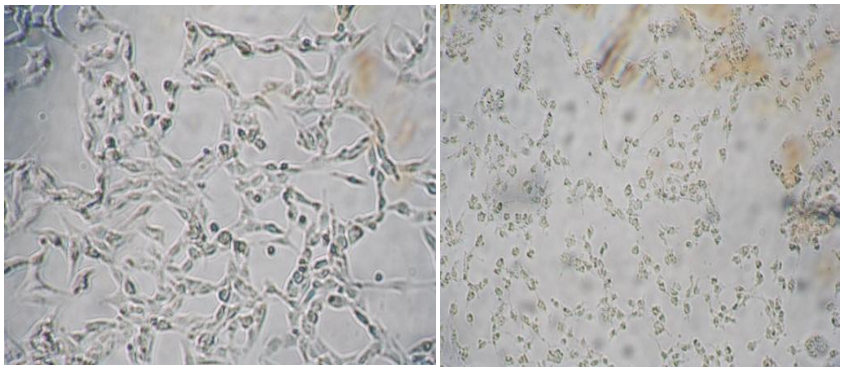
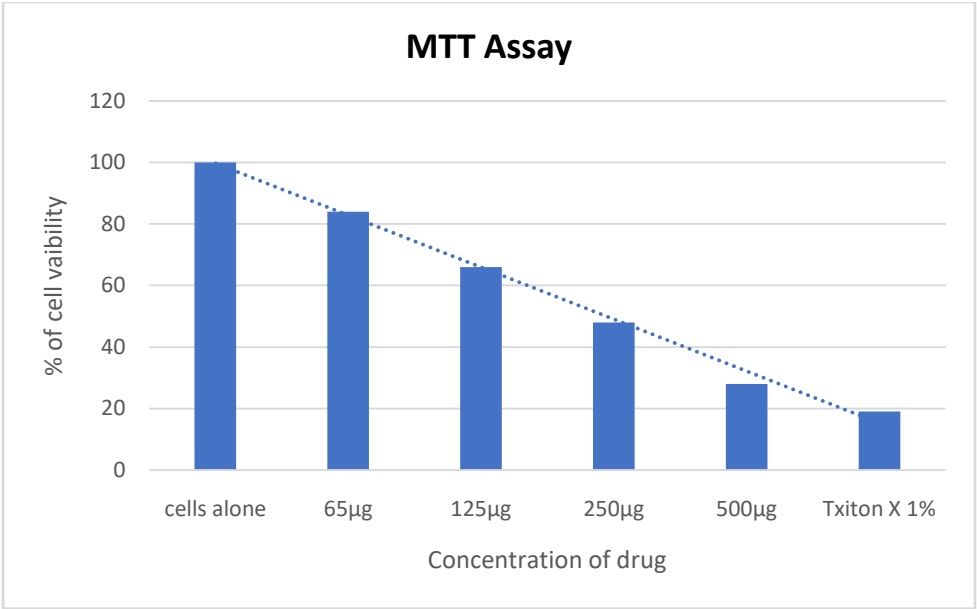
DNA damages by comet assay, and apoptotic morphological changes by AO/EtBr-staining method.



Control cells **Treated cells**

Figure 1 - Photograph shows morphology of AGS Gastric cancer cell line (a) control and (b) treated cells



Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

Figure 2 - Effectof C.decandra fraction on % of cell viability (24 h) in control and *C.decandra*treated AGS cells

Effect of *C.decandra* generates the intracellular ROS

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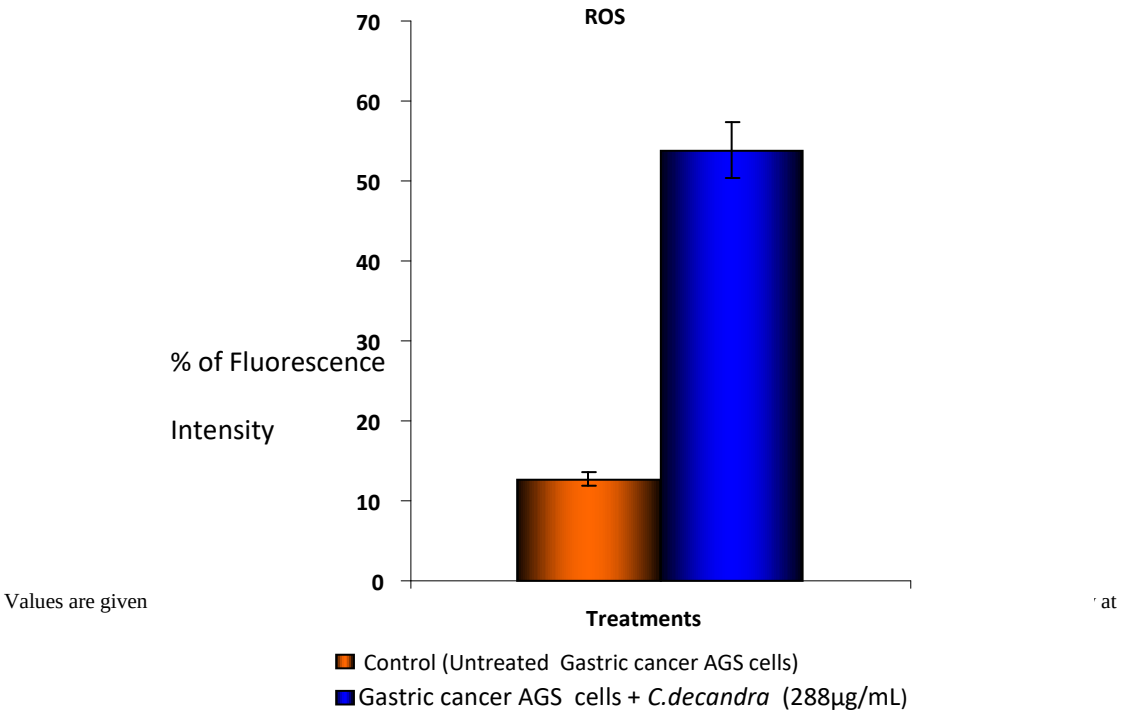
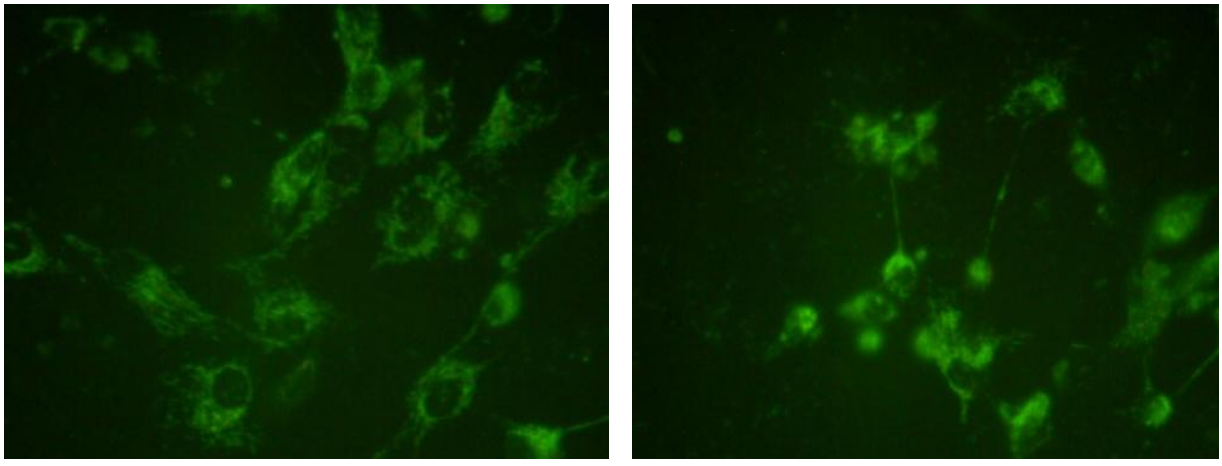
Levels of ROS in control and *C.decandra*-treated cells are depicted in Fig. 3&4 (a-b) *C.decandra*treated cells significantly increased ROS level in AGS cells. At the concentrations 288 µg/mL *C.decandra* show significant ROS production in AGS cells. Spectrofluorimetric analysis revealed that the *C.decandra* treatment significantly increased ROS level in AGS cells.The level of apoptotic morphological changes *control and C.decandra* treated cells



Values are given at

Figure 3 - *C.decandra* enhances ROS levels in AGS -Gastric cancer cells



Control ***C.decandra* (288µg/ mL)**

Annals of R.S.C.B., ISSN: 1583-6258, Vol. 25, Issue 6, 2021, Pages. 20422 – 20448

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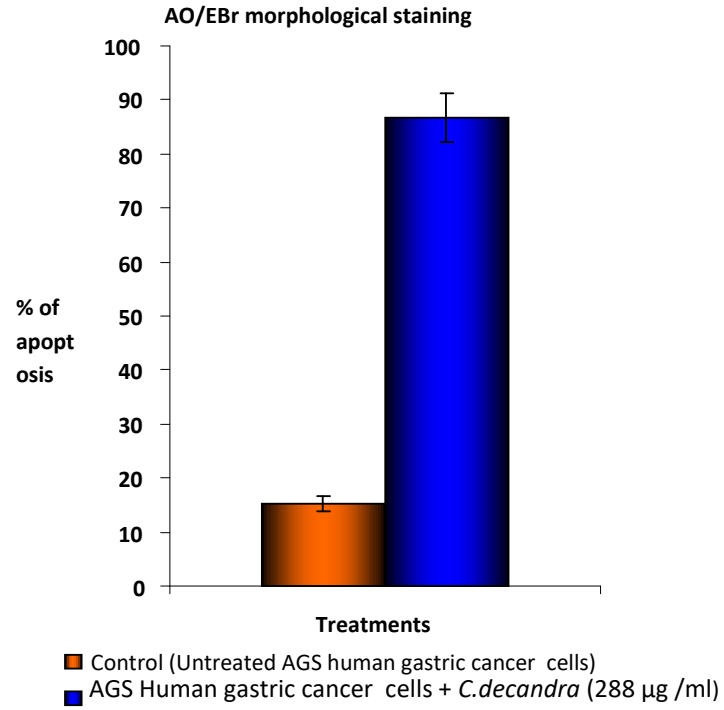
Figure 4 - Effect of *C.decandra* on ROS levels in (a) control *and (b)C.decandra* treated AGS gastric cancer cells

Effect of *C.decandra*on apoptotic morphological changes in AGS cells

Fig. 5 &6 (a-b) show the effect of *C.decandra*extract on apoptotic morphological changes. The fluorescence microscopic observationof the untreated and treated cancer cells. Untreated cancer cells appeared in green (AO stained) whereas *C.decandra* treated cells appeared in red/orange (EtBr stained) that revealed the presence of apoptotic cells. Fig. 5&6 shows the percentage of apoptosis. *C.decandra* concentrations of 288µg/mL increased apoptotic cells levels to 90 %, respectively, when compared to the control group



P<0.05 (DMRT)

Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at <0.05 (DMRT)

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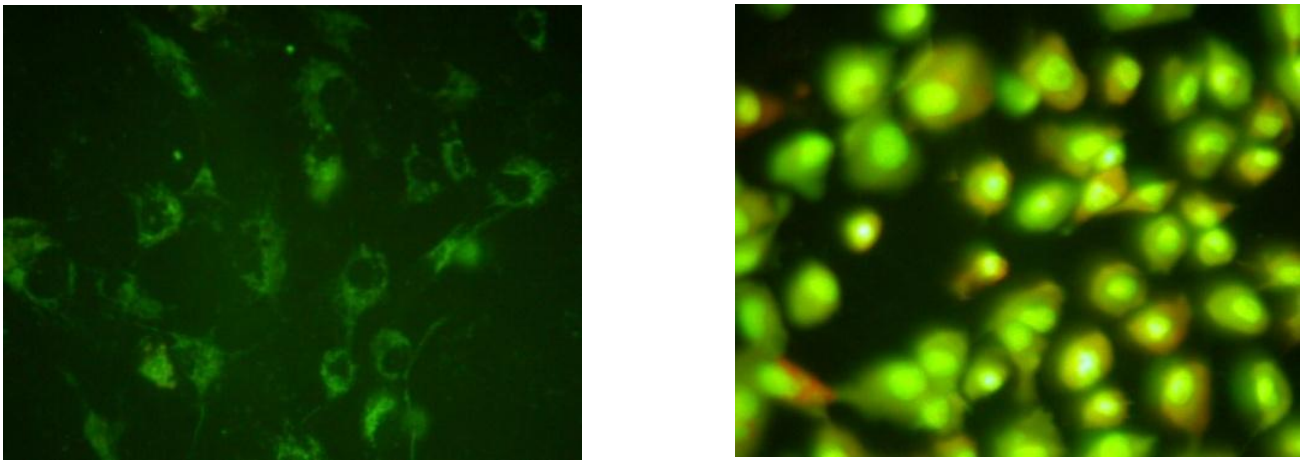
Figure 5 - Effect of *C.decandra* on apoptotic morphological changes in human Gastric cancer AGS cells



Control ***C.decandra*** **(288 *µ*g/ ml)**

Figure 6 - Effect of *C.decandra* (24 h) on apoptotic morphological changes in (a) control and (b) *C.decandra* treated AGS Gastric cancer cells

Effect of *C.decandra* generates the mitochondrial membrane potential

Changes in mitochondrial membrane potential in control and *C.decandra*-treated cells are depicted in Fig.7 and 8 (a-b) *C.decandra* treated cells significantly increased mitochondrial depolarization in AGS cells. The present result shows accumulation of Rh-123 dye in the control group and the Rh-123 accumulation was decreased in *C.decandra*, treated cells as the membrane potential decreased. At the dose of 288µg/mL of *C.decandra* showed the highest level of mitochondrial depolarization in AGS cells. Polarized mitochondria were markedbyorange-red fluorescence and depolarized mitochondria were marked by green fluorescence(Fig. 7&8).

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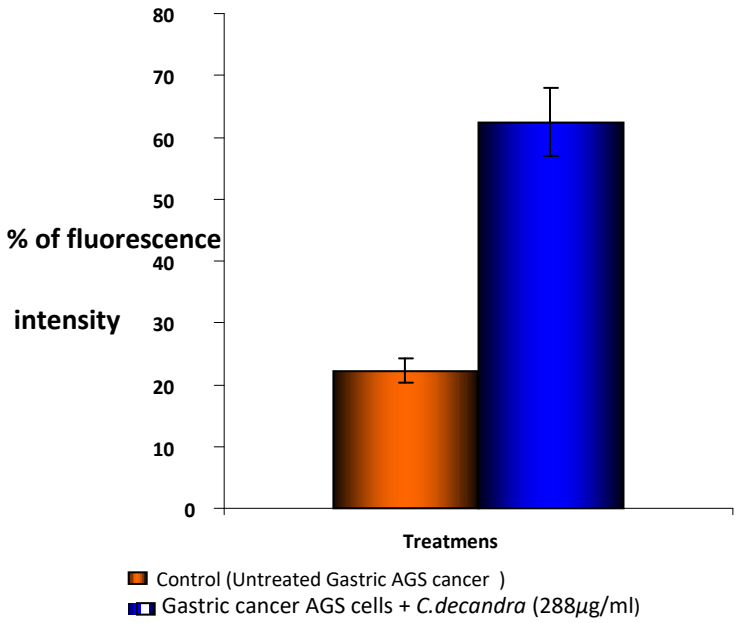
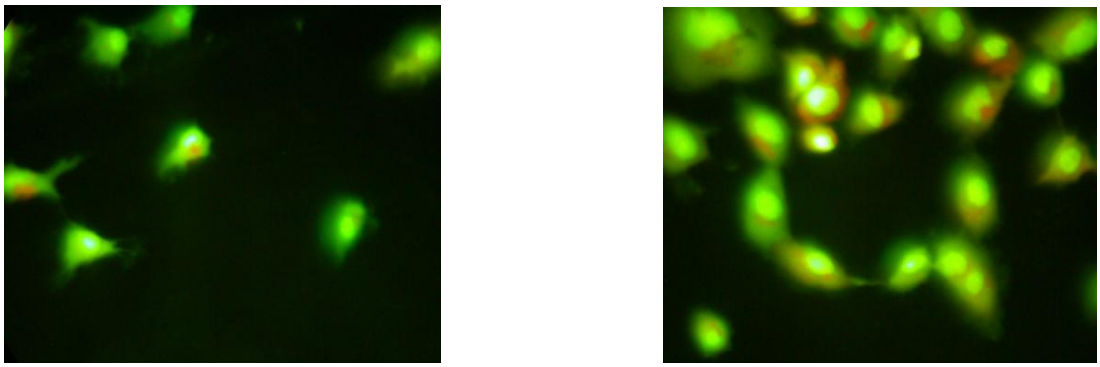


Figure 7 - Effect of *C.decandra* on mitochondrial membrane potential in AGS cell



control ***C.decandra*** **(288 µg /ml)**

Figure 8 - Effect of *C.decandra* (24 h) on mitochondrial membrane potentials (a) control and (b)*C.decandra* treated cells

DNA fragmentation analysis

Present study examined the extent of nuclear DNA fragmentation by agarose gel electrophoresis and the results are shown in Fig 9&10 (a-b). DNA ladder pattern was observed after the treatment of AGS cell *C.decandra* at concentration 288 µg/mL. This suggested that these *C.decandra*caused DNA fragmentation characteristic of apoptotic

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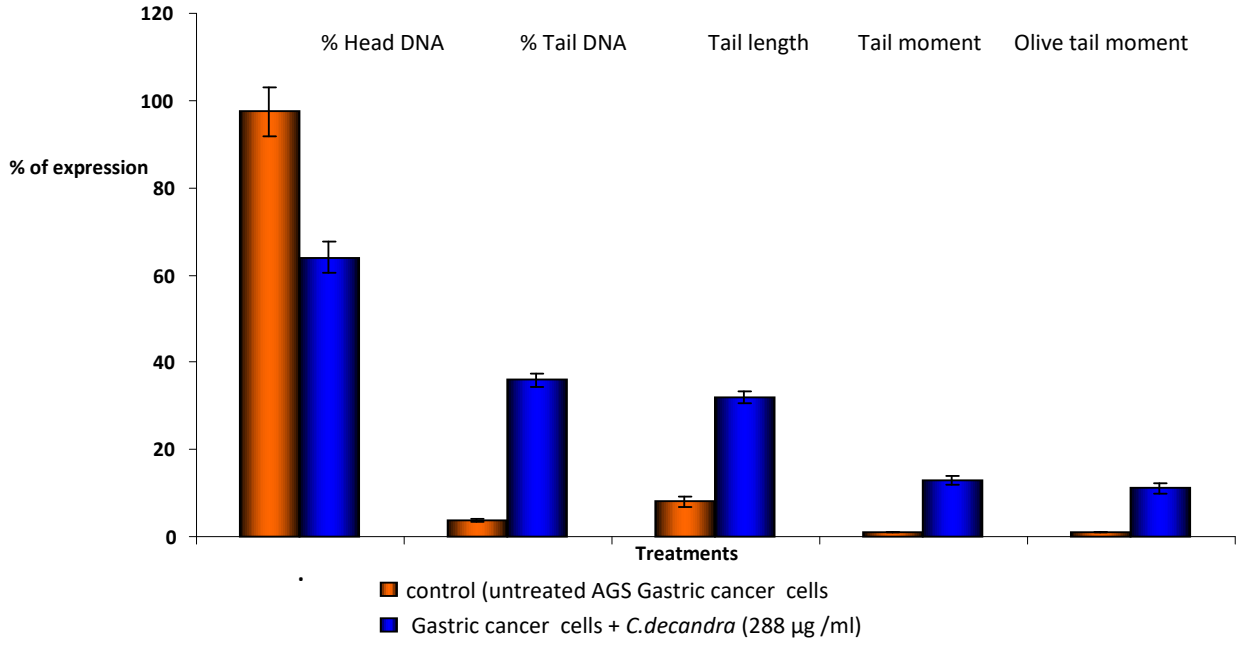
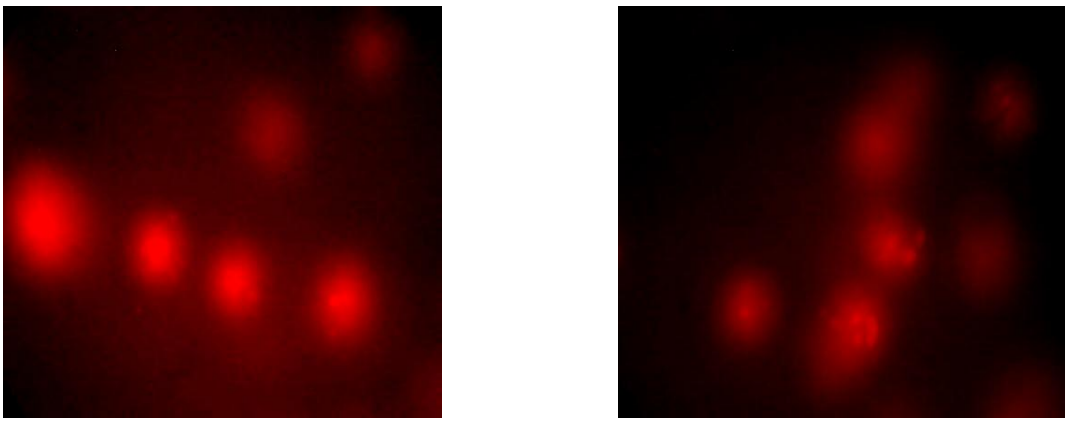
process with the generation of multiple DNA fragments and induced apoptosis in AGS cancer cell at different incubation duration. Similarly, observed increased oxidative DNA damage (% Tail DNA, % Tail length, Tail moment, and olive tail moment(Fig 9 &10)



Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT)

Figure 9 - Effect of *C.decandra* on DNA damage(Comet Assay) in AGS cells



Control ***C.decandra*(288 µg/ ml)**

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Figure 10 - Effect of *C.decandra*onDNA damage(Comet Assay) on (a) control and *(b)C.decandra* treated cells

Changes in the levels of lipid peroxidative markers and the activities of enzymatic antioxidants

In this study observed the levels of lipid peroxidative markers, such as TBARS, CD and LHP in control and *C.decandra*treatedcells (Fig. 11). *C.decandra* treated cells increased the levels of lipid peroxidation in AGS cells. The results show that *C.decandra* enhances lipid peroxidative markers such as TBARS, CD, and LHP in AGS gastric cancer cell line (Fig 11). The level of enzymatic antioxidants such as SOD, CAT, and GPxare are depicted in **Fig.12**. *C.decandra* (288 μg/mL) treated cells significantly decreased the activities of SOD, CAT, and GPx in AGS cells. At the concentration of *C.decandra* (288μg/mL) treated cells significantly decreased enzymatic activities when compared with control in AGS cells. In this study, the effect of *C.decandra*glutathione levels in *C.decandra*-treated cancer cells was examined. Levels of GSH in control and *C.decandra* cells are showed in **Fig.13.** The treatment of *C.decandra*treated cells (288 μg/mL) decreased GSH levels in AGS cells compare to control cells. These data suggest that *C.decandra*fraction exhibits potent anticancer effect in AGS cell line, and that it may be used as an anticancer agent.

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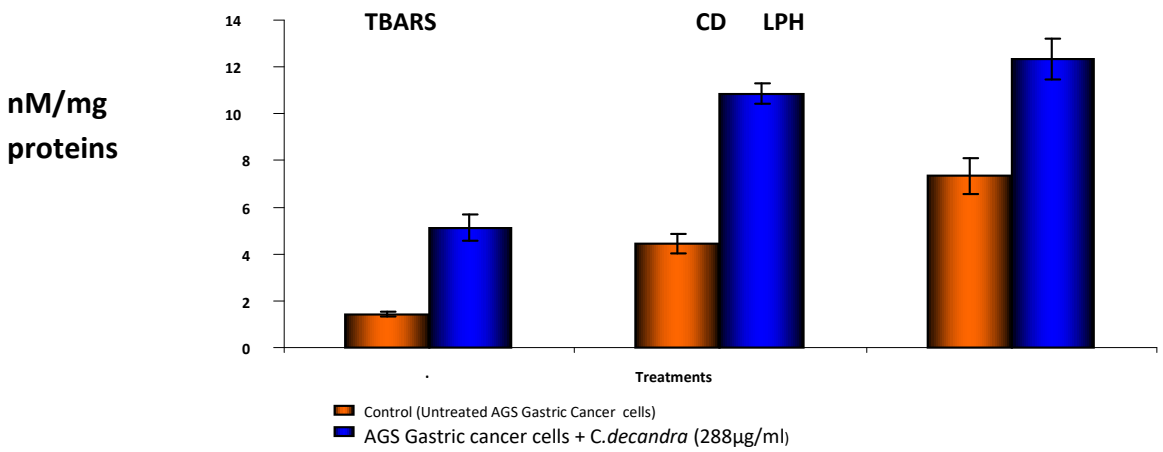
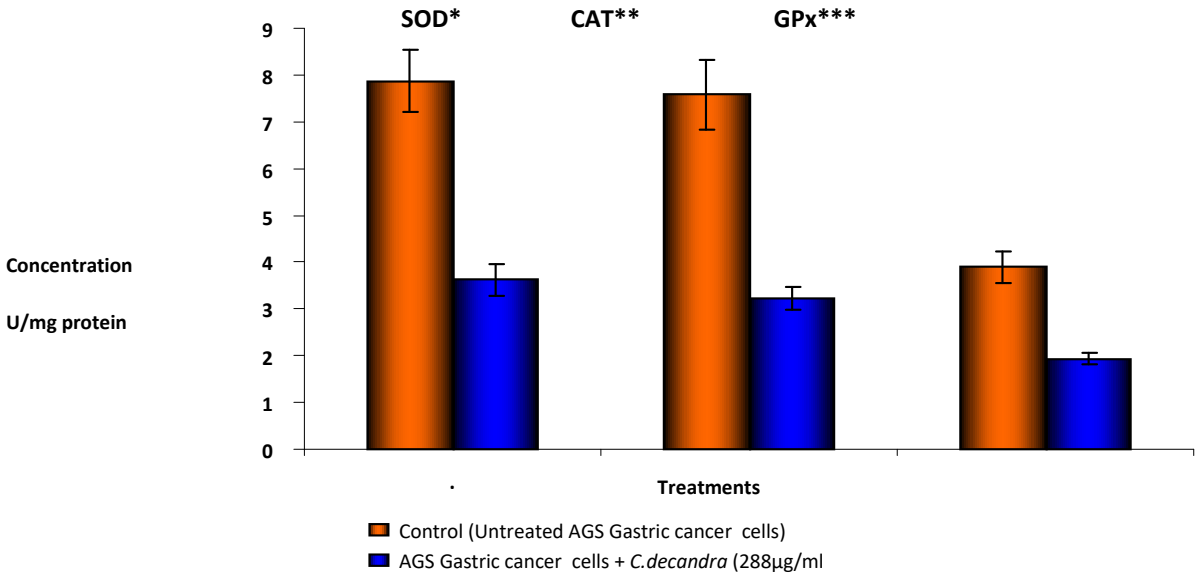


Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

Thiobarbituric acid reactive substances (TBARS), conjugated dines (CD), and lipid hydro peroxide (LHP)

Figure 11 - Effect of *C.decandra*on the levels of TBARS, CD and LPH in control and *C.decandra* treated AGS cancer cells



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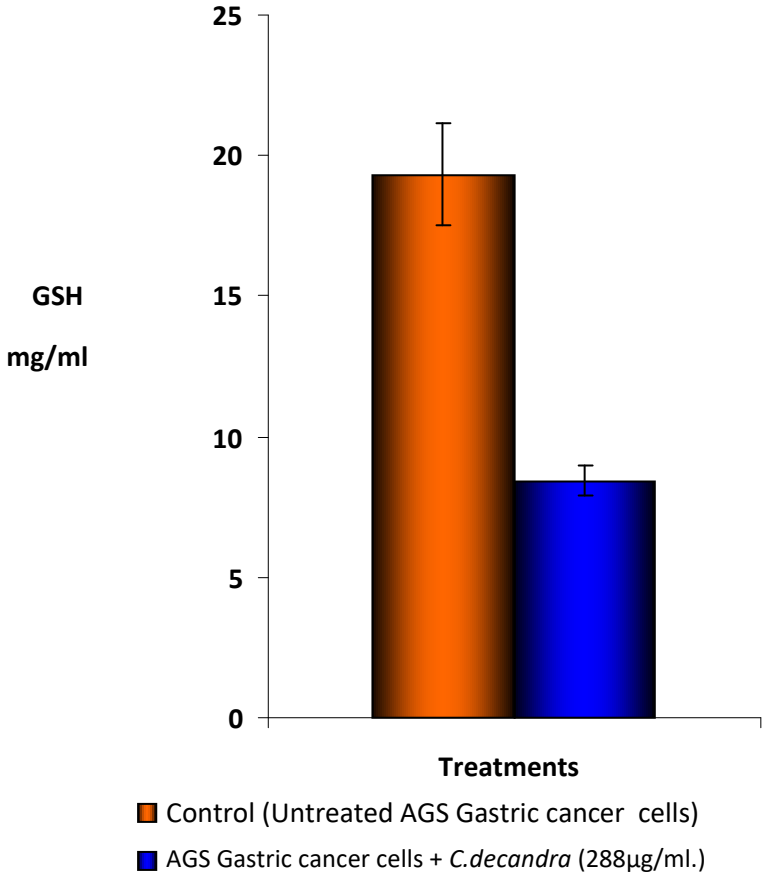
* Enzyme activity required for 50% inhibition of nitroblue tetrazolium reduction in oneminute, ** µmol of hydrogen peroxide consumed per minute, *** µg of glutathione consumed per minute. Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

Figure 12 - Activities of SOD, CAT and GPx in control and *C.decandra* treated AGS cells



Values are given as mean ± S.D. of six experiments in each group. Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

Figure 13 - Levels of GSH in control and *C.decandra* treated AGS cells

Discussion

Cancer is a dreadful human disease increasing with changing life style, nutrition, and global warming. Cancer treatments do not have potent medicine and the currently available drugs

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causing side effects in some instances. In this context, the natural products derived from medicinal plants have gained significance in the treatment of cancer. According to the WHO, 80% of the world's population primarily those of developing countries rely on plant-derived medicines for the health care[13] and Marine flora such as bacteria, actinobacteria, cyanobacteria, fungi, microalgae, seaweeds, mangroves and other halophytes extremely constitute over 90% of the oceanic biomass. They are taxonomically diverse, largely productive, biologically active and chemically unique offering a great scope for discovery of new anticancer drugs and also rich in medicinally potent chemicals predominantly belonging to polyphenols and sulphated polysaccharides(SitharangaBoopathy*et al.,* 2011).In present study, the cytotoxicity effect of *C.decandra* in AGS cells by MTT assay was observed. After 24 h, potentially inhibited the growth of cells than compared to*C.decandra* lower concentrations (10, 20, 50,100 and 200 μg/mL). The 300 μg/mL could greatly inhibit the cell growth. At 288 μg/mL concentration of *C.decandra* exhibited pro-oxidant property. The cytotoxic activity increased in a dose-dependent manner after 48 h. The IC_{50} values calculated by plotting cytotoxicity curves were *C.decandra* 10 μg/mL, 100μg/mL and 300 μg/mL respectively which were < 300 μg/mL and hence the extract can be considered as 'active' according to National Cancer Institute's guidelines (Boyd, 1997) . Hence, in this present study revealed that*C.decandra* indicated good IC_{50} values as 288 μg/mL.

Mangrove species treatment caused a rapid increase of intracellular ROS in AGS cells **(Figure 3&4).** Significant increase was noticed in the ROS levels was reached in 288 µg/mL *C.decandra* treated cells. The increased ROS levels during *C.decandra* treatment might be due to its prooxidant property. The prooxidant activity of plant extract has been already confirmed by several researchers (Karadag *et al.*, 2007; Oya *et al.*, 2011). ROS such as O_2•- and H_2O_2 is recognized to induce SOD, CAT and GPx. Higher activities of antioxidant

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Received 20 August 2021; Accepted 10 September 2021.

enzymes have been observed in malignant tumours compared to controls(Kumaraguruparan*et al.,* 2002).This reaction has an autocatalytic character since $O_2^{\bullet-}$will oxidize the phenolic compound to regenerate the semiquinone and H_2O_2.

C.decandra treatment significantly increased mitochondrial depolarization in AGS cells. The present results also demonstrated that cytochromes c was released from mitochondria into the cytoplasm, followed shows accumulation of Rh-123 dye in the control and the Rh-123 accumulation were decreased in *C.decandra* treated cells as the membrane potential decreased. Among all the doses, 288 μg/mL of *C.decandra* showed high level of mitochondrial depolarization in AGS cells. The present results along with previous reports suggest that anti cancer activity of mangrove plants may be influenced by mangrove and mangrove associated plant and that might be the reason for increased cytotoxicity observed in marine plants treated cells (Mashjoor*et al.,*2016; Thirunavukkarasu*et al.,* 2015)The further observed changes in the mitochondrial membrane potential in *C.decandra* treated and control cells. Mitochondrion is one of the most important organelles in regulating cell death as well as a maker in apoptosis(Hildeman*et al.,* 1999). The mitochondria of normal cells pump H^+ from intimal ground-substance to the outside of the endomembrane when electrons are in respiratory chain. This caused a transmembrane potential Rh 123 were served to determine the alteration of mitochondrion membrane potential. The uptake of the cationic fluorescent dye, rhodamine 123, has been used for the estimation of mitochondrial membrane potential. Rhodamine 123 is the membrane permeable dye specific to mitochondria. Accumulation of rhodamine 123 was observed in the mitochondria of control cells (**Figure 7&8).** Whereas the *C.decandra* treated cells showed no uptake of rhodamine 123. This indicated that mitochondrial membrane potential has been altered during *C.decandra* treatment. AGS cell death induced by *C.decandra* may be attributed to mitochondrial toxicity as the

Received 20 August 2021; Accepted 10 September 2021.

mitochondrial membrane potential collapsed before apoptosis ensued. Phenoxyl radical-induced ROS levels might contributes to mitochondrial toxicity. Previously, Vijayavel*et al.,* 2006 studies showed that *R.apiculata* extract alters the mitochondrial membrane potential and induce mitochondrial collapse. Procyanindin, another dietary polyphenol, induce apoptosis in PC3 cells by altering mitochondrial membrane potential(Ramachandran *et al.,* 2008; Deepika*et al.,* 2019).**Figure 9&10** shows significant DNA damage in *C.decandra* treated AGS cells. Previous report mangrove plant to be known to enhance oxidative DNA damage in cancer cells(Demple and Harrison 1994; Asha Poorna*et al.,* 2013). In present study, tumour cells showed response to the treatment of *C.decandra* but more pronounced response was seen in higher doses of *C.decandra*. Previously, phytochemicals has been reported to induce DNA damage in cervical cancer cells by reactive oxygen species generation(Srinivas *et al.*,2004). Since cancer cells possess centrally acidic region *C.decandra* could not be able to act as antioxidant and most phenolics act as prooxidant in cancer cells. The increased DNA damage in *C.decandra* treated cells be due to its prooxidant effect. A large number of natural compounds have shown cytotoxic effects in a variety of situations i.e. either alone or together with radiation (Vermund and Gollin 1968). Flavonoids autooxidize in aqueous medium and may form highly reactive free radicals. Marine plants may act as substrate for peroxidases and other metalloenzymes, yielding quinone type prooxidants(Deepika*et al.,* 2018). This might be the reason for increased oxidative DNA damage (% tail DNA, tail length, tail moment, Olive tail moment) observed in *C.decandra* treated cancer cells.

In the present study, estimated the effect of *C.decandra* on lipid peroxidation indices i.e. TBARS, CD and LHP in AGS cells. In this study observed significant increase in lipid peroxidation indices in *C.decandra* treated cancer cells. Mangrove plants have been reported

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to stimulate hydroxyl radical formation and reduce ferrylmyoglobin(Goh and Jantan, 1991). Which suggests their potential prooxidant action. Mangrove plant extract stimulated the formation of hydroxyl radicals in reaction mixture containing H_2O_2, $FeCl_3$ and EDTA. Thirunavukkarasu*et al.*,2011*)* reported showed prooxidant activity of mangrove extract on Cu^{2+} induced low density lipoprotein oxidation. Similarly ascorbic acid is known to switch from anti to prooxidant activity, depending on its concentration and on the presence of free transition metal ions (Bendrich, *et al.,* 1986).Many studies suggested that antioxidant enzymes are critical in protecting against tumour promoting agents. Interestingly, cell malignancy or transformation is often accompanied by a decrease in activity of antioxidant enzymes like superoxide dismuatase (SOD), catalase (CAT) and glutathione peroxidase (GPx), which increases the cell sensitivity to prooxidant compounds(Sergediene*et al.*,1999). The susceptibility of tumour cells to radiation is associated with decreased activities of antioxidant enzymes (Dal-Pizzol*et al.*,2003). In this study, observed by decreased activities of antioxidant enzymes i.e. SOD, CAT and GPx in *C.decandra* treated cancer cells. Many studies reported that mangrove plant and their compounds decreased the antioxidant enzymes like SOD, CAT and GPx in experimental models (SitharangaBoopathy*et al.,* 2011; Thirunavukkarasu*et al.,* 2011).High concentration of cellular antioxidants is an important determinant of oxidative cancer cell death. In the present study noticed prominent decrease of GSH levels in cancer cells treated with *C.decandra*. Previous studies shows that mangrove plant depletes intracellular antioxidants thereby induced cancer cell death (Reddy and Ratna Grace, 2016). *C.decandra* extracts slightly reduce cellular viability and induce minimal DNA damage in AGS cell lines as assessed. GSH is ubiquitous in aerobic tissues, and although it is not a nutrient, it is synthesized from sulfhydryl-containing amino acids and is highly important in intermediary antioxidant metabolism(Vad*et al.*, 2009).

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The proliferation of AGS cell lines was significantly inhibited by *C.decandra*. The inhibitory effect was observed after 24 h of incubation. Among the different treatments 288 μg/mL of *C.decandra*showed only 50% cell viability. Polarized mitochondria are marked by orange-red fluorescence and depolarized mitochondria are marked by green fluorescence. Control cells appeared orange-red in colour whereas the *C.decandra*treated cells appeared has green in colour. Rhodamine 123 is a lipophilic cationic dye that enters only live cells and stain mitochondrial DNA and hence the live cell mitochondria appeared orange-red in colourunder blue emission. Among all the doses tested 288 μg/mL of *C.decandra* showed high level of mitochondrial depolarization in AGS cells. The DNA damage was significantly increase in the levels of observed in *C.decandra*treated cells when compared with control. The DNA fragmentation was significantly increase in the treatment cell compared to control. Similar results also were in the lower traces (10, 25 , 50, 150 250μg/mL) of *C.decandra* . *C.decandra* treated resulted in significant reduction in thiobarbituric acid reactive substrances (TBARS) and Lipid hydroperoxides (LPH) levels. Further, antioxidant like superoxide dismutase(SOD). Catalase(CAT), glutathione peroxidise(GPx), reduced glutathione(GSH), Vitamin-C(Vit-C) and Vitamine-E wre normalised in sqaulene treated cells. So it may be good and potential anti gastric cancer drugs in feature. Further research need to be explored to study the bioactive compounds of *C.decandra* and for the successful implication of them as a potent therapeutic tool against novel cancer drug candidate.

Conclusion

It is evident from the present study that *C.decandra* derived fraction offers a remarkable protection against AGS gastric cancer cells. Mangrove isolated berberine initiate cancer cell death by decreasing cell proliferation, antioxidant status and mitochondrial membrane

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potential and by increasing intracellular ROS, lipid peroxidation and apoptosis in gastric cancer (AGS) cancer cell. It would be a potent anti gastric cancer drug.

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