Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 464-468
Received: 17-07-2017
Accepted: 18-08-2017

Tania Chakraborty
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Amrita Pal Basak
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Ankita Mridha
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Priya K Gopal
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Santanu Paul
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Correspondence
Santanu Paul
Laboratory of Cell and Molecular Biology, Department of Botany, Centre of Advanced Study; University of Calcutta, Kolkata, India

Anti-cancer and anti-oxidant potential of Indian carpet weed *Glinus oppositifolius* (L.) Aug. DC

Tania Chakraborty, Amrita Pal Basak, Ankita Mridha, Priya K Gopal and Santanu Paul

Abstract
Glinus oppositifolius (L.) Aug. DC. is an angiospermic weed of the family Molluginaceae. Delving through available literature it is found that this plant has been long used in traditional cultures and multiple ethnic groups in India and other South East Asian countries for medicinal purposes. This study aims to quantify the antioxidant potential of this plant and to check whether it has any cytotoxic effect on cancer cell lines. Methanolic fraction extracted from dried leaves has been used for the study. DPPH radical scavenging, inhibition of lipid peroxidation, Superoxide scavenging assay, nitric oxide scavenging assay, total phenol content and total flavonoid content assays were performed and it showed that the methanolic extract of this plant exhibits moderate levels of antioxidant capacity. Methanolic extract of *Glinus oppositifolius on* different human cancer cell lines reveals enhanced anti-proliferative efficacies as compared to normal peripheral blood mononuclear cells (PBMCs). *Glinus oppositifolius* therefore makes a potential repository for identifying novel anti-cancer drugs.

Keywords: Anticancer, Antioxidant, *Glinus oppositifolius*, Scavenging Assay, MTT assay

1. Introduction
Cancer is many diseases grouped into one umbrella term, all of whom share one common characteristic above all- the unnatural growth of cells. Cancer is an ancient disease and nowadays, the incidence of malignant cancer and cancer mortality is on the rise. According to the International Agency of Research for Cancer (IARC), the rise will be about 50% in the next 20 years [1]. In low to middle income countries, a quarter of disease related deaths are caused due to cancer [1, 2].
An estimate has been put forward that approximately 50% of cancer patients in the United States utilize medicinal agents that are derived from different plant parts or plant nutrients either exclusively or with conventional therapeutic procedures such as chemotherapy or radiation treatment [3]. Since the 1940s a range of anticancer drugs have been introduced in the market and about 73% of them could be traced to be derived from natural products [4]. Various reports show that phytochemicals affect various aspects of cell cycle progression in cancer and undoubtedly play a role in gene expression, apoptosis and metabolism [5]. Even in combination chemotherapy phytochemicals have been seen to play a major part [5, 6]. Several studies have documented the anticancer effects of groups of phytochemicals like quercetin, catechins, reseveratrol, and curcumin that work synergistically with various cancer drugs and/or other plant compounds [5]. As a matter of fact, up to the start of the new millenium, researchers have identified anticancer potentials of thousands of plants [7, 8] and pharmaceutical companies have investigated more than 20,000 plants as possible sources of anticancer drugs [9].
For the present study, the plant *Glinus oppositifolius* was chosen. It is an angiospermic weed of the family Molluginaceae. The plant has been widely used in traditional medicine for curative purposes and also features prominently in ethnobotanical accounts. The plant is widely available across India, and other areas panning the South Eastern Asian region and also across in Mali, a country to the west corner of Africa.
Several classes of phytochemicals like phenols and flavonoids take part in scavenging reactive oxygen species in plants, for which they are collectively termed as “antioxidants”. In living organisms, the process of cellular oxidation constantly goes on during metabolic processes and produces energy necessary to keep the organism functioning normally. However, as a by product of this process, reactive oxygen species (ROS) and other free radicals are formed. These are highly reactive *in vivo* and also highly unstable due to the presence of a single electron in the outer orbital which can attack other biomolecules [10]. Excess ROS reacts with essential biomolecules like proteins, lipids and DNA and destabilizes these biomolecules.

Since ROS production beyond a normal threshold is harmful for the organism, natural quenchers ("antioxidants") are present which maintain the balance of reactive oxygen species [11]. These compounds have also been studied to see if they have any effects on cancer cells. Antioxidants have had a chequered history concerning their reported ability to prevent or treat cancer. Depending on the structure, dose, target molecule, and environment, phenols or flavonoids may stimulate or inhibit the oxidative damage processes to biomolecules [12]. Reports show that they can behave as either antioxidants or pro-oxidants [13, 14] and that the inhibitory potency displayed on the growth and proliferation of certain malignant cells *in vitro* is strongly dependent on their structural characteristics [13, 15].

The cell lines used for this study are REH, Hep-G2 and MOLT-4. REH is a human B cell precursor leukemia cell line, and it was initiated from the peripheral blood of a 15 year old North African girl with acute lymphoblastic leukemia (ALL at first relapse) in 1973. This line differs by its chromosome markers and by the absence of EBNA and the presence of EBV receptors and other B markers. REH cells are presently utilized for active immunotherapy of patients with acute lymphoid leukaemia. Hep-G2 is a human liver cancer cell line and the cells are adherent, epithelial-like cells growing as monolayers in small aggregates. The cells were derived from the liver tissue of a 15 year old Caucasian male with hepatocellular carcinoma. MOLT-4 is a human T cell lymphoblast leukemia cell line with a hypertetraploid chromosome number.

2. Materials and methods

2.1. Chemicals

All chemicals used were of the analytical grade. RPMI 1640 (Sigma Aldrich), FBS (Invitrogen), Penicillin and Streptomycin (Sigma-Aldrich), MTT 3-(4, 5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich), DMSO, Phosphate Buffer Saline, Gallic acid (Merck), Sodium Carbonate (Merck), Folin Ciocalteau (Merck), Quercetin (SRL), Sodium nitrite (Himedia), Aluminium Chloride (Merck), Sodium hydroxide (SRL), 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (SRL), Methanol (Merck), Nitroblue tetrazolium (SRL), NADH (SRL), Sodium phosphate buffer, PMS (SRL), Phosphate buffer saline, Acetic acid (Merck), 0.5% TBA, Thiobarbituric acid, n-Butanol (Hi-media), Phosphate buffer.

2.2. Collection & preparation of methanolic extract of plant

Whole plant was collected in the monsoon months of July-August, 2016. The plants were then sun-dried for a week and the leaves were ground into fine powder. 10 gram of *Glinus oppositifolius* powder was mixed with 80 mL of hexane to form hexane extract for 72 hours at room temperature and then filtered. A sufficient amount of hexane extract was unavailable for *Glinus oppositifolius*. The residue was air dried to evaporate out hexane and then 175 mL methanol was added. The mixture was shaken continuously for 10-15 minutes at regular intervals and then filtered after a week. The filtrate was evaporated to dryness and powdery methanol fraction was collected, weighed, marked and stored in a microcentrifuge tube wrapped with parafilm at 4 °C for future use. From the methanolic fraction, a master stock of 5 mg/mL was prepared with DMSO, which was also marked and capped with parafilm and kept for use.

2.3. Cell lines and Normal PBMCs

REH, B Acute Lymphocytic cell line; Hep G2, liver cancer cell line; MOLT 4, T Acute Lymphocytic Leukemia cell line. Cell lines were cultured at $1x10^5$ cells/mL in RPMI 1640 supplemented with 2mM L-glutamine, 10% (v/v) heat-inactivated FBS, 10 U/mL penicillin and streptomycin and maintained in humidified 5% CO_2 incubator (HF-90). Normal PBMCs were separated from normal healthy donors [16].

2.4. measurement of anti-oxidant capacity

2.4.1. determination of total phenol content of plant extract

Total phenol content of the methanol extract was evaluated according to the method of Singleton VL & Rossi JA, 1965 [17] and Mridha *et al*., 2017 [18] using Folin-Ciocalteau reagent. The phenolic content was estimated from a standard curve prepared with gallic acid. Equal volume of crude extract and 0.2 N Folin-Ciocalteau reagent were added and incubated for 3 minutes. 10% Sodium carbonate was added, the reaction mixture was vortexed and incubated for 40 minutes in dark at 25°C. O.D. was measured spectrophotometrically at 760 nm. The experiment was performed in duplicates at each concentration. Total phenol content methanolic extract of *Glinus oppositifolius* was determined as mg of gallic acid equivalents (GAE) per gram of methanolic extract.

2.4.2. Determination of total flavonoid content of plant extract

Total flavonoid content was evaluated according to the method of Kamtekar *et al*. (2014) [19] and Mridha *et al*., 2017 [18]. The flavonoid content was estimated from a standard curve prepared with quercetin. 1 ml of aliquot was mixed with 4 mL of distilled water and 5% sodium nitrite solution was added. The mixture was incubated for 5 minutes and then 10% aluminum chloride and 1 M NaOH were added. The volume of the reaction mixture was made up to 10 mL with distilled water and vortexed for 10 seconds. Absorbance was measured at 510 nm. The experiment was performed in duplicates at each concentration. Total flavonoid content methanolic extract of *Glinus oppositifolius* was determined as mg of Quercetin equivalents per gram of methanolic extract.

2.4.3. Determination of dpph radical scavenging activity of plant extract

DPPH scavenging activity of methanolic extract of *Glinus oppositifolius*was evaluated according to the method of Bloiss *et al*. 1958 [20] and Mridha *et al*., 2017 [18]. 800 µL of DPPH solution (0.1mM/mL in methanol) was added to 200 µL of extract (at concentrations of 10, 20, 40, 60, 80, 100 µg/mL). The mixtures were vortexed and incubated in the dark at room temperature for an hour. Absorbance was measured at 517 nm. The experiment was performed in duplicates at each concentration. The percentage scavenging of DPPH by the crude methanolic extract was calculated according to the following formula:

Percentage DPPH radical scavenging = $[(Absorbance_{Control}-Absorbance_{Sample})/ Absorbance_{Control}] \times 100$

2.4.4. Determination of percentage inhibition of lipid peroxidation by plant extract

Thiobarbituric acid-reactive species (TBARS) assay [21] was used to measure the percentage inhibition of lipid peroxidation by crude plant extract with some modifications. 20% v/v egg homogenate and 0.1mL of plant extract were added to a test tube and the mixture was made up to made up

to 1ml with distilled water. 0.07M FeSO4 was added and the reaction mixture was incubated for 30 min. Acetic acid (pH 3.5), TBA in 1% SDS, reaction mixture was vortexed and heated at 95°C for an hour. Butanol was added to each tube after the reaction mixture cooled down and the tubes were centrifuged at 3000 rpm for 10 min. The upper organic layer was separated out and the absorbance was measured at 532 nm.

Percentage inhibition of lipid peroxidation by the crude methanolic extract was calculated according to the following equation:

$[(Absorbance_{Control}\text{-}Absorbance_{Sample})/ Absorbance_{Control}] \times 100$

2.4.5. Determination of superoxide anion scavenging activity of plant extract

Superoxide anion scavenging activity was estimated according to the methods of Kakkar *et al*. 1984 [22] and Mridha *et al*. (2017) [18]. Equal volume of plant methanolic extract at various concentrations were taken and to each 50 µM Nitroblue tetrazolium (NBT), Phosphate buffer, Nicotinamide adenine dinucleotide (NADH), 10 µM PMS were added. The reaction mixture was vortexed and incubated at 37°C temperature in dark for five minutes. The absorbance of the reaction mixture was measured at 560 nm. The following formula was used to determine the superoxide radical scavenging activity.

Percentage superoxide radical scavenging = $[(Absorbance_{Control}\text{-}Absorbance_{Sample})/ Absorbance_{Control}] \times 100$

2.5 cell viability assay

Methanolic fraction of *Glinus oppositifolius* was dissolved in DMSO. Cells were seeded at $5x10^4$ cells/well in a 96-well plate with three different concentrations- 10µg/mL, 25 µg/mL, 50µg/mL, and 100µg/mL of the methanolic fraction and observed at 24 hours time point employing MTT cell viability assay [16]. $5x10^4$ cells were incubated in duplicate in a 96-well plate at different concentrations of methanolic extract of *Glinus oppositifolius* in a final volume of 200µL for 24 hrs at 37°C. MTT solution (5mg/mL) was added to each well three hours before the completion of incubation time, Cell viability was spectrophotometrically measured at 560 nm. 100% lysis was obtained by lysing the cells in 5% SDS lysis buffer, and absorbance of 100% lysed cell sample was also measured at 560 nm.

The percentage of cell viability was calculated as mentioned below:

$\%$ cell viability= $(O.D._{sample}\text{-}O.D._{100\%lysis})/ (O.D._{0\%lysis}\text{-}O.D._{100\%lysis}) *100$

3. Results & discussion

3.1 total phenol content

Total phenolic content of methanolic extract of *Glinus oppositifolius* was determined with the Folin-Ciocalteau reagent and is expressed as miligram of gallic acid equivalents per gram of methanolic extract. From the equation y= 0.0001x+0.1757 (x = gallic acid concentration, y = absorbance of *Glinus oppositifolius* methanolic extract, obtained from the standard curve of gallic acid, the total phenol content is calculated to be 205±19.091 mg of gallic acid equivalents/g dry weight of tissue.

3.2 total flavonoid content

Total flavonoid content of methanolic extract of *Glinus oppositifolius* was determined with the Aluminium chloride assay and is expressed as miligram of quercetin equivalents per gram of methanolic extract. From the equation y= 0.00037x+ 0.09462 (x = quercetin concentration, y = absorbance of *Glinus oppositifolius* methanolic extract, obtained from the standard curve of quercetin, the total flavonoid content is calculated to be 32.908±11.084 mg Quercetin equivalent per gram dry weight of tissue.

3.3 DPPH radical scavenging assay

DPPH radical scavenging assay was carried out with four different concentrations of methanolic extract of *Glinus oppositifolius* –100 µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL. Results show that the percentage of scavenging increases in a linear direction with the increase of concentration. However, the scavenging is moderate even in the highest concentration, with none of the concentrations being able to cross 50% scavenging, thereby not achieving EC50. This portrays the moderate antioxidant capacity of the crude methanolic extract of *Glinus oppositifolius*.

3.4 inhibition of lipid peroxidation

Determination of inhibition of lipid peroxidation by TBARS assay using egg yolk as source of lipid is a well known method for quantifying antioxidant activity. Different concentrations of methanolic fraction of *Glinus oppositifolius* were tested and the antioxidant activity was seen to increase proportionally to the linear increase of extract concentration. The EC50 value was calculated to be 124 µg/mL. Thus the assay shows that the methanolic extract of *Glinus oppositifolius* shows to a moderate to good antioxidant potential in terms of inhibiting lipid peroxidation across the range of concentrations studied.

3.5 superoxide radical scavenging assay

A range of concentrations across two logarithmic scales of concentration were used for this assay. It was seen that even with increase of concentration in a geometric progression, the increase in radical scavenging activity was not drastically enhanced, and the range of scavenging was from 27% to 45% for a range of concentrations from 10µg/mL to 1000 µg/mL. The quantification of superoxide radical scavenging is a measure of antioxidant capacity and for the methanolic extract of *Glinus oppositifolius*, the antioxidant capacity is moderate across the range of concentrations studied.

3.6 cell viability assay

The methanolic fraction of *Glinus oppositifolius* shows a moderate anti-proliferative effect on the leukemic cell line REH when subjected to a cell viability test at the time point of 24 hours. The IC_{50} value of REH, MOLT-4 & HEP-G2 is 31.2µg/mL, 82.45µg/ml and IC_{50} value of HEP-G2 could not be reached. The anti-cancer effect of *Glinus sp*. obtained from MTT data shows methanolic extract of *Glinus* is most potent against REH >MOLT-4>HEP-G2.

4. Conclusion

Our studies show that methanolic extract of *Glinus oppositifolius* possesses moderate antioxidant activity in the assays performed to quantify the antioxidant potential of this plant. The methanolic fraction shows an appreciable cytotoxic effect on the leukemia cell line REH, and MOLT 4 compared to normal PBMC cells, which proves that the methanolic fraction of this plant possesses anticancer attributes. Results of MTT of *Glinus* methanolic extract on HEP-G2 cells show low anti-proliferative effects at the tested concentrations. From the data obtained so far it can be concluded that

methanolic fraction of *Glinus oppositifolius* possess appreciable anti-cancer efficacies and can be tapped for isolation of potent anti-cancer drugs however more studies on control and other cancer cell lines are required to be have a better understanding on the anti-cancer effects of the methanolic fraction of *Glinus oppositifolius.* Data taken together reveals that *Glinus oppositifolius* could be included in the hunt for specific, potent and safer anti-cancer drugs in future.

5. Conflict of interest

The authors report no conflict of interest.

6. Author contributions

SP designed the study. APB collected and dried the plants. TC prepared the methanolic extract and performed the laboratory tests with the help of AM and APB. Cell viability assays were performed by PKG. SP and TC analyzed data and wrote the manuscript. All authors approved the final manuscript.

7. Acknowledgement

The authors are indebted to UGC-UPE and UGC-CAS programme at the Department of Botany, University of Calcutta for financial support.

Figure legends

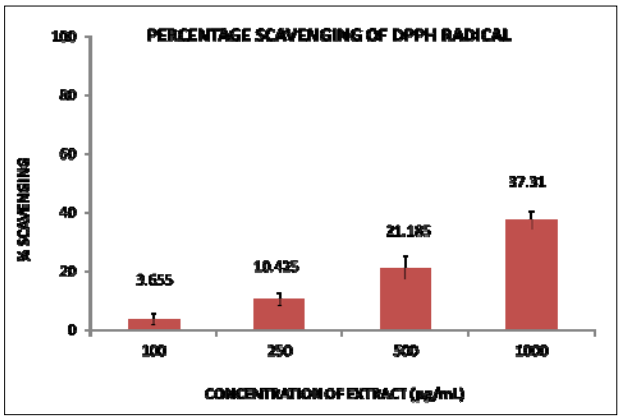

Fig 1: DPPH radical scavenging activity of methanolic extract of *Glinus oppositifolius* at different concentrations ranging from 100 µg/mL to 1000 µg/mL. The bars indicate mean ± standard deviation.

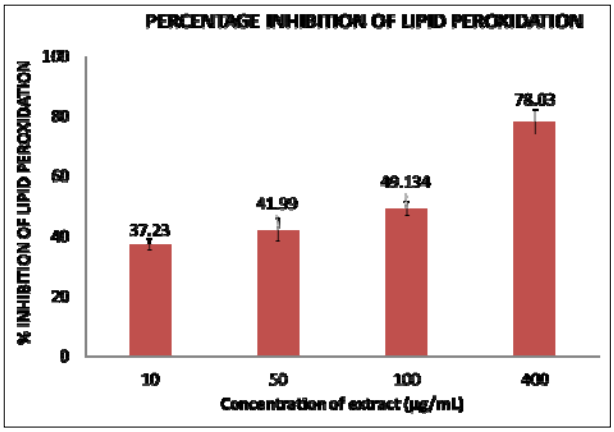

Fig 2: Percentage inhibition of lipid peroxidation activity of methanolic extract of *Glinus oppositifolius* at different concentrations ranging from 10 µg/mL to 400 µg/mL. The bars indicate mean ± standard deviation.

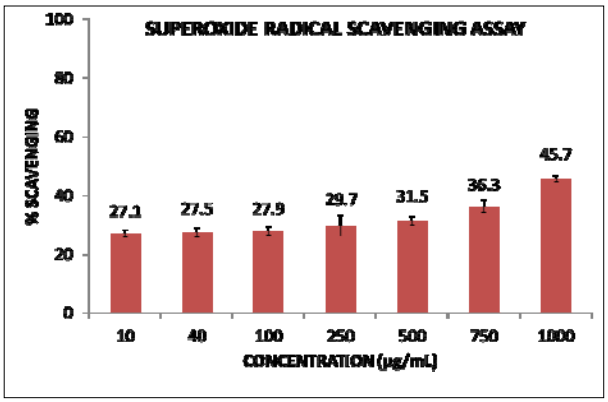

Fig 3: Percentage scavenging of superoxide radical by methanolic extract of *Glinus oppositifolius* at different concentrations ranging from 10 µg/mL to 1000 µg/mL. The bars indicate mean ± standard deviation.

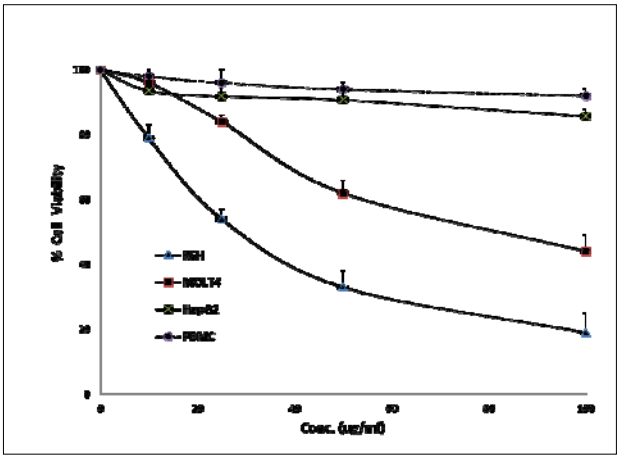

Fig 4: Cytotoxic effect of methanolic extract of *Glinus oppositifolius* on three different cancer cell lines REH, MOLT-4 & HEP-G2 versus normal PBMCs. The extracts were treated at concentrations 0-100µg/ml and MTT performed after 24hrs time point.

8. References

1. Stewart BW, Wild CP. World Cancer Report. International Agency of Research for Cancer (IARC), 2014.
2. Piniewski-Bond J, Celestino PB, Mahoney MC, Farrell CD. A cancer genetics education campaign: delivering parallel messages to clinicians and the public. J Cancer Educ, 2003; 18:96-99.
3. Gutheil WG, Reed G, Ray A, Dhar A. Crocetin: an Agent Derived from Saffron for Prevention and Therapy for Cancer. Curr Pharm Biotechnol. 2012; 13(1):173-179.
4. Newman DJ, Cragg GM. Natural products from marine invertebrates and microbes as modulators of antitumour targets. Curr Drug Targets. 2006; 7:279-304.
5. Hema Iswarya S, Doble M. Potential synergism of natural products in the treatment of cancer. Phytother Res. 2006; 20:239-49.
6. Liu RH. Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. Am J Clin Nutr. 2003; 78:517S-20S.
7. Mukherjee AK, Basu S, Sarkar N, Ghosh AC. Advances in cancer therapy with plant based natural products. Curr Med Chem. 2001; 8:1467-1486.
8. Akter R, Shaikh JU. Cytotoxic activity screening of Bangladeshi medicinal plant extracts. J Nat Med. 2014; 68:246-252.

9. Larkin T. Herbs are often more toxic than magical. FDA Consum. 1983; 17:4-11.
10. Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR. Antioxidant activity and free radical-scavenging potential of *Pithecellobium dulce* Benth seed extracts. Free Radical and Antioxidants, 2011; 2(2):37-43.
11. Masoko P, Eloff JN. Screening of twenty-four South African *Combretum* and six *Terminalia* species for antioxidantactivites. African Journal of Traditional, Complementary and Alternative Medicines. 2007; 4(2):231-239.
12. Khan NS, Ahmad A, Hadi SM. Antioxidant, Prooxidant Properties of Tannic Acid and Its Binding to DNA. Chemico- Biol. Interac. 2000; 125:177-189.
13. Sergediene E, Jonsson K, Szymusiak H, Tyrakowska B, Rietjens ICM, Cenas N. Prooxidant Toxicity of Polyphenolic Antioxidants to HL-60 Cells: Description of Quantitative Structure- Activity Relationships. FEBS Lett. 1999; 462:392-396.
14. Cao G, Sofic E, Prior RL. Antioxidant and Prooxidant Behaviour of Flavonoids: Structure-Activity Relationships. Free Rad. Biol Med. 1997; 22:749-760.
15. Castellucio C, Paganga G, Melikian N, Bolwell GP, Pridham J, Sampson J. Antioxidant Potential of Intermediates in Phenylpropanoid Metabolism in Higher Plants. FEBS Lett. 1995; 368:188-192.
16. Gopal PK, Paul M, Paul S. Curcumin Induces Caspase Mediated Apoptosis in Jurkat Cells by Disrupting the Redox Balance, Asian Pac J Cancer Prev, 15(1):93-100.
17. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Vitic. 1965; 16:144-158.
18. Mridha A, Nandi C, Pal R, Paul S. Studies on few fresh water green algal species reveals *Spirogyra triplicata* as the repository of high phenolicand flavonoid content exhibiting enhanced anti-oxidantproperty, Journal of Pharmacognosy and Phytochemistry. 2017; 6(4):1291-1297.
19. Kamtekar S, Keer V, Patil V. Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. J appl pharm sci. 2014; 4(09):061-065.
20. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199-1200.
21. Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. Food Chemistry. 2005; 90:727-733.
22. Kakkar P, Das B, Vishwanathan PN. A modified spectrophotometric assay of superoxide dismutase, Indian J Biochem Biophys. 1984; 21(2):130-132.