



IN VITRO ESTIMATION OF ANTIOXIDANT ACTIVITY OF SELECTED MEMBERS OF POACEAE AND CYPERACEAE FAMILY

Garima Gupta^{1*} and Sumit Kumar²

¹Research Scholar, Shri Venkateshwara University, Gajraula, Amroha, U.P., India.

²Associate Professor, School of Life Science, Shri Venkateshwara University, Gajraula, Amroha, U.P., India.

*Corresponding Author: Garima Gupta

Research Scholar, Shri Venkateshwara University, Gajraula, Amroha, U.P., India.

Article Received on 07/03/2018

Article Revised on 27/03/2018

Article Accepted on 17/04/2018

ABSTRACT

The vegetable oils are an essential component of every household, but due to their short term stability the need to enhance their shelf life for long term storage is an important area of research. The vegetable oils which are fortified with synthetic antioxidants are shown to have significantly higher stability in terms of oxidation but their side effects at higher concentrations limit their use in food products. This study aims to determine the effect of various Poacea and Cyperaceae family species extract on lipid oxidation inhibition of vegetable oils. First the five selected plant species were screened for their antioxidant activity. This was done by making water extract of all these plants and then testing them for their phenolic content, flavonoid content and reducing capacity. The species showing maximum antioxidant activity was combined with all the other species to check the antioxidant activity of the combinations. The leaf extract of combination of *Vetiveria zizanioides* and *Cynodon dactylon* showed the highest amount of antioxidant activity. Thus this extract combination can be used as a food supplement in order to enhance the shelf life of various edible oils.

KEYWORDS: *Vetiveria zizanioides*, Phenolics, polyphenolic, nutraceutical.

ABBREVIATIONS

TCP: Total phenolic content

TFC: Total flavonoid content

GAE: Gallic acid equivalent

RE: Rutin equivalent

FW: Fresh weight.

1. INTRODUCTION

Reactive oxygen species (ROS) are produced in metabolic processes to generate energy and metabolites or to produce defense against pathogenic microorganisms.^[1] It has been justified that ROS produced in the human body may cause oxidative damage related with many degenerative disorders like cardiovascular disease, cancer and neurodegenerative diseases for example Parkinson's and Alzheimer's diseases.^[2] It has been identified that there is a reciprocal association between consumption of fruits, vegetables, whole cereal grains etc. and mortality from degenerative disorders. The connection between more consumption of fruit, grain products, vegetable etc and lower risk of chronic disorders can be associated to antioxidant protection. Antioxidants are compounds that slow down or inhibit the oxidation of cellular targets. They show their effects by scavenging ROS or inhibiting their production.^[3,4,5] Polyphenols are considered as the most

varied and broad class of natural compounds which are probably the major natural antioxidants. The maximum number of active phenolic compounds extracted from higher plants is flavonoids and phenolics acids. These compounds present a broad spectrum of biological and chemical reactions including radical scavenging characteristics.

The Poaceae and Cyperaceae families are considered as an important group of useful plants. Species from these families have bioactive compounds like flavonoids (e.g. Cglycosides of apigenin, tricetin, luteolin), phenolic acids (e.g. ferulic acid, *p*-hydroxybenzoic acid, caffeic acid) and triterpenes, saponins, sterols. Many of the grass species have showed therapeutic effect (e.g. strong antioxidant characteristics) and have been significantly effective in the healing of inflammations and sclerosis.^[6,7] As per the studies, the phenolic and antioxidant properties of leaves of *Vetiveria zizanioides* have not been investigated yet. The contents of phenolic compounds and their antioxidant activity in the selected grass species have been poorly investigated. Therefore, testing their antiradical properties is of interest, primarily in order to find new sources of natural antioxidants. The objective of this study was to determine the antioxidant activity and estimation of total phenolic and flavonoid

content of the selected plant species as a primary screening tool for selecting the plant with maximum antioxidant properties. Plant species selected for determining antioxidant activity are: Nutgrass (*Cyperus rotundus*), *Paspalidium flavidum*, *Dactyloctenium aegyptium*, Durva (*Cynodon dactylon*) Grass and *Vetiveria zizanioides*. Nut-grass or Motha is generally seen growing as weed in garden, lawn, fields and waste lands. It appears like grass and has tuberous roots underground. It is a perennial plant. Its blackish tuberous roots have typical smell due to existence of essential oil. These rhizomes roots have a lot of medicinal properties and are used in Ayurveda for healing of diseases since ancient times. They are utilized fresh and dried. For medicinal use the dried powder or decoction of roots are prescribed. *Paspalidium flavidum* is found to be a wonderful fodder and the grain is collected and consumed in times of drought and scarcity. The leaves and roots are considered to be a bit cyanogenetic. The leaves show antiseptic properties and their paste is utilized topically in cutaneous infections. It is also utilized for over indulgence and cyanogenetics. It is consumed by horses and cattle and it generates a large amount of grain that has been consumed by man in times of scarcity. *Dactyloctenium aegyptium* is a grass which has a lot of uses. It is mostly utilized as fodder and enjoyed by all groups of ruminants. In semi-arid areas it provides economical annual pastures as well as wonderful hay. It is also used for silage.^[8] The seeds may be fed to poultry or utilized to produce alcoholic beverages, and are consumed by humans in periods of food scarcity. They contain ethno-medicinal characteristics and can be utilized as a fish poison.^[9] It is present all over Indonesia, Central America, and the northern part of South America, Africa, the Arabian Peninsula, India, South-East Asia and Australia. In Hinduism, Durva (Doob) is considered as the most sacred plant, only followed by Tulsi. It is a grass utilized in many religious occasions since Vedic times as well as for therapeutic purpose in Indian Ayurvedic medicine system. On Durvashtami fast (in Bhado), Hindu women pray Durva and keep fast. The circular rings made from this grass are worn during Hom, Puja and Yagya. Durva is also offered to Lord Ganesh while praying. It is among the ten auspicious herbs that make up the group Dashapushpam in Ayurveda. There has to be some reasons, why this plant is thought to be so divine and

inseparable from various Hindu occasions. May be, this is because of its medicinal and therapeutic capacity. This holy plant has a lot of medicinal characteristics. Its roots and leaves contain a lot of therapeutic properties. Durva is present all over India and also in many regions of world as common grass. Durva plant has vitamin C, cartone, palmitic acid, triterpenoides, alkaloids, crude proteins, carbohydrates and mineral constituents, oxides of magnesium, phosphorous, calcium, sodium and potassium. Durva can be utilized both internally and externally for therapeutic purpose. *Vetiveria* which is commonly called as *Khus* grass is a perennial grass of Indian origin. It is found all over the plains, lower hills, on riverbanks and rich marshy soil across India. *Khus* grass has a lot of uses from household to therapeutic. Its roots are utilized as curtains, dried stems to manufacture brooms and dried plant to make roofs. Mostly the roots are utilized for therapeutic purposes which are both aromatic and have sedative impact on nervous system and is also utilized to treat intestinal parasites, fever, skin diseases and poisonous stings. *Vetiver* oil is extracted from roots of plant. It is used for flavouring *Khus Sharbat* and in making of cosmetics, perfumes, soaps etc.^[10]

2 MATERIAL AND METHOD

2.1 Sample Preparation

Fresh leaves of all the plants were shade dried and coarsely powdered in grinder. 40 gm of this powder was soaked in 200ml distilled water and kept for extraction for 48 hours at room temperature. This residue was filtered and concentrated by rotary vacuum evaporator. Final concentration was made 0.2mg/ml. The combinations were made by first estimating individual samples and then the sample showing maximum activity was combined with all other samples in equal proportion.

2.2 Determination of Total Phenolic Content

The determination of TPC of the grass extract was performed by using Folin-Ciocalteu reagent.^[11] Briefly, 1ml of extract was prepared with 1.8 ml of Folin-Ciocalteu reagent (10 fold diluted) and kept for 5 min at 25 degree Celsius. Later 1.2 ml of 15% Sodium Carbonate was added to the reaction mixture and kept for 90 min at RT and the absorbance was measured at 765nm. The concentration of the TPC was determined as mg of Gallic acid equivalents (GAE) per gm FW.

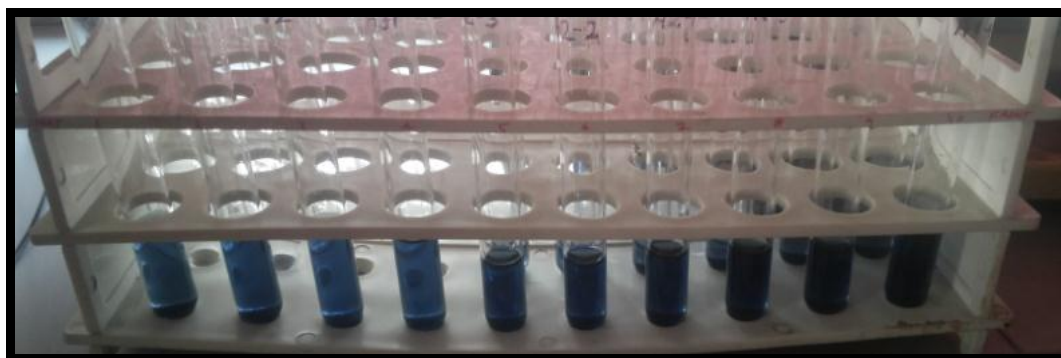


Figure 20: Samples of Total Phenolic Content Assay.

2.3 Determination of Total Flavonoid Content

TFC of grass extract was determined by using the aluminium chloride colorimetric method (Chavan JJ et al, 2013). Briefly, 0.5 ml of the extract, 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1

M potassium acetate and 2.8 ml of distilled water were mixed for 5 min by vortexing. Reaction mixture was kept at RT for 30 min and the absorbance was measured at 415 nm. The results were expressed as mg of rutin equivalents (RE) per gm FW.

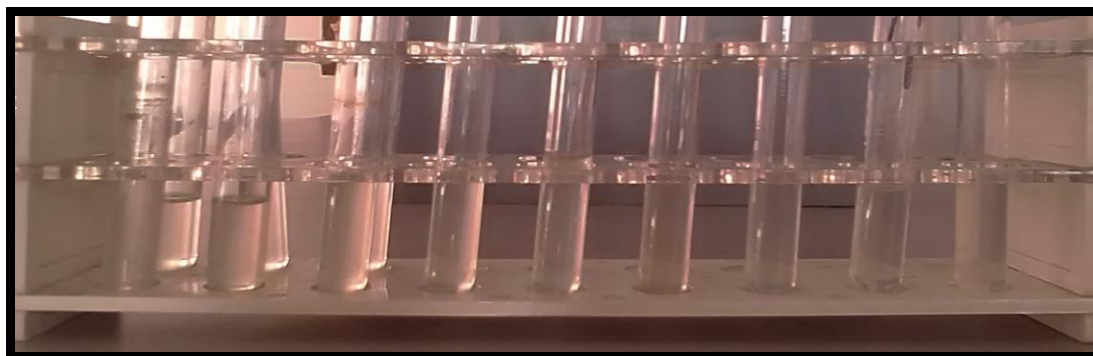


Figure 21: Samples of Total Flavonoid Content Assay.

2.4 Determination of Reducing Power

The reducing power was determined by the method Athukorala et al (2006) (8). 1 ml sample of different concentration were taken. 1ml 0.2M sodium phosphate buffer pH 6.6 was added to each sample. 1ml of 1% potassium ferricyanide was added and incubated at 50°C

for 20 minutes. 1ml of 10% TCA (W/V) was added and then the samples were centrifuged at 2000 rpm for 10 minutes, 2.5ml of upper layer was taken and mixed with 2.5 ml DW. 0.5 ml of 0.1% fresh ferric chloride was added and the readings were taken at 700 nm.



Figure 22: Samples of Reducing Power Assay.

3 RESULTS

3.1 Total Phenolic Content

The variation of the total phenolic content over time for various concentrations of all the samples is presented in Table 4. TPC of the extract was expressed as mg GAE/g of dry weight. Phenolic content GAE is increasing with increasing concentration in all the samples and it is

observed that the content is remarkably high in the combination of *Vetiveria zizanioides* and *Cynodon dactylon* sample. The maximum concentration of 7.24 mg/gm GAE of dry weight is observed at 0.2 mg/ml concentration of combination of *Vetiveria zizanioides* and *Cynodon dactylon* extract.

Table 4: Estimation of Total phenolic content. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

Sample conc. (mg/ml)	A	B	C	D	E	CA	CB	CD	CE
0.08	1.665 ± 0.05	1.85 ± 0.08	1.97 ± 0.02	1.71 ± 0.06	1.6 ± 0.07	3.345 ± 0.08	2.67 ± 0.02	2.51 ± 0.06	1.9 ± 0.07
0.12	2.355 ± 0.04	2.25 ± 0.03	2.38 ± 0.04	1.86 ± 0.05	1.8 ± 0.04	4.495 ± 0.03	4.21 ± 0.04	3.25 ± 0.05	2.3 ± 0.04
0.16	2.82 ± 0.05	3.15 ± 0.06	3.30 ± 0.03	2.3 ± 0.04	2.1 ± 0.03	4.89 ± 0.06	5.48 ± 0.03	4.225 ± 0.04	2.92 ± 0.03
0.2	3.335 ± 0.06	3.96 ± 0.05	4.20 ± 0.05	3.2 ± 0.03	2.8 ± 0.05	5.47 ± 0.05	7.24 ± 0.05	4.425 ± 0.03	3.32 ± 0.05

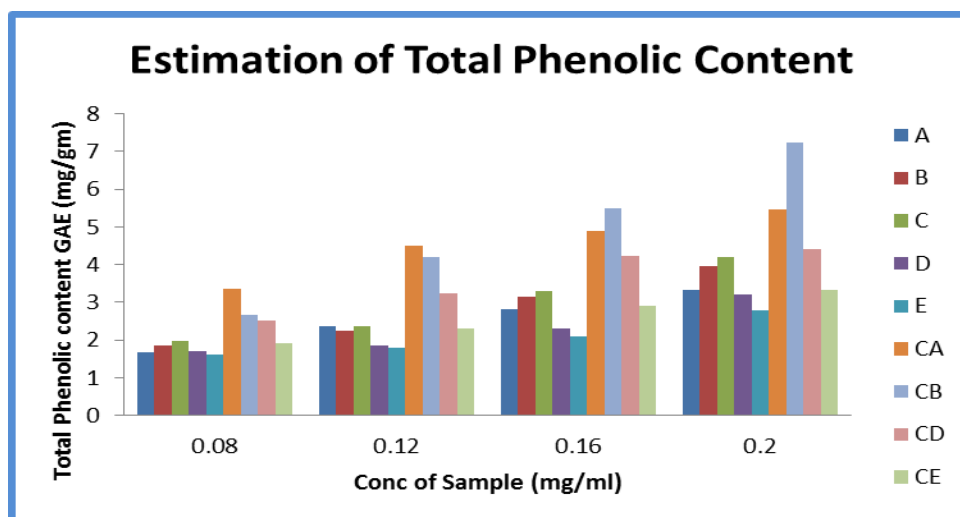


Figure 23: Estimation of Total phenolic content. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

3.2 Total Flavonoid Content

The variation of the flavonoid content over time for various concentrations of all the samples is presented in Table 5. Flavonoid content of the extract was expressed as mg RE/g of dry weight. Total Flavonoid content (TFC) is increasing with increasing concentration in all

the samples and it is observed that the content is highest in the combination of *Vetiveria zizanioides* and *Cynodon dactylon*. The maximum concentration of 10.8 mg/gm RE of dry weight is observed at 0.2 mg/ml concentration for combination of *Vetiveria zizanioides* and *Cynodon dactylon* extract.

Table 5: Estimation of Total Flavonoid content. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

Sample conc. (mg/ml)	A	B	C	D	E	CA	CB	CD	CE
0.08	3.6 ± 0.03	1.80 ± 0.03	2.82 ± 0.05	0.98 ± 0.06	1.54 ± 0.07	4.0 ± 0.03	4.4 ± 0.05	1.2 ± 0.06	3.6 ± 0.07
0.12	6.11 ± 0.08	3.92 ± 0.05	4.62 ± 0.07	3.23 ± 0.03	3.42 ± 0.02	6.58 ± 0.05	7.6 ± 0.07	5.17 ± 0.03	5.64 ± 0.02
0.16	6.72 ± 0.05	6.17 ± 0.07	6.79 ± 0.03	5.92 ± 0.07	5.82 ± 0.05	8.82 ± 0.07	10.0 ± 0.03	7.14 ± 0.07	5.88 ± 0.05
0.2	7.6 ± 0.02	7.91 ± 0.04	8.20 ± 0.04	7.56 ± 0.05	7.4 ± 0.03	10.0 ± 0.04	10.8 ± 0.04	9.2 ± 0.05	7.6 ± 0.03

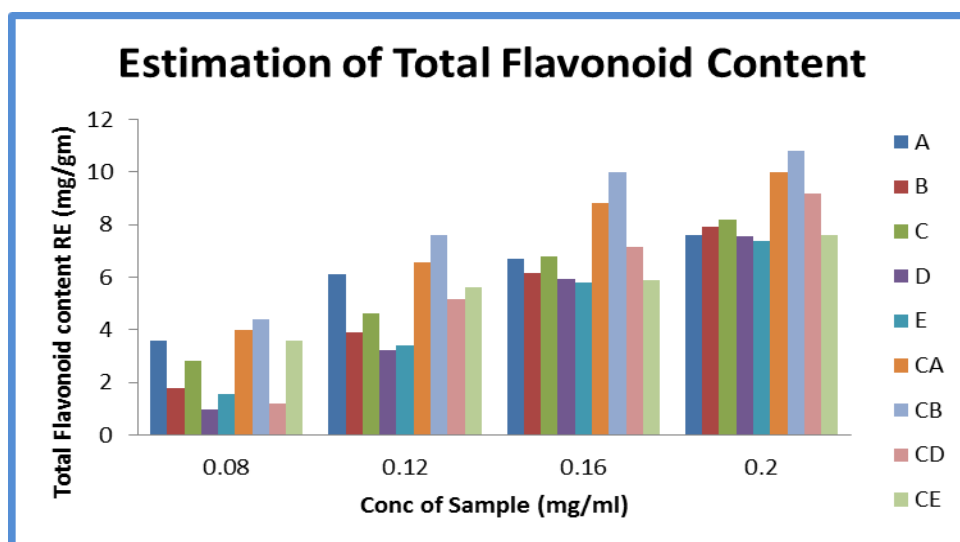


Figure 24: Estimation of Total Flavonoid content. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

3.3 Reducing Power Assay

The variation of the reducing activity over time for various concentrations of all the samples is presented in Table 6. Reducing activity is concentration dependent and increases with increasing concentration in all the samples. It is observed that the combination of *Vetiveria*

zizanioides and *Cynodon dactylon* showed the highest activity among all the samples. The maximum activity of 0.578 is observed at 0.2 gm/ml concentration of combination of *Vetiveria zizanioides* and *Cynodon dactylon* extract.

Table 6: Estimation of Reducing Power Activity. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

Sample conc. (mg/ml)	A	B	C	D	E	CA	CB	CD	CE
0.08	0.124 ± 0.07	0.133 ± 0.05	0.156 ± 0.04	0.152 ± 0.03	0.189 ± 0.08	0.213 ± 0.05	0.198 ± 0.04	0.265 ± 0.03	0.219 ± 0.08
0.12	0.268 ± 0.05	0.248 ± 0.08	0.256 ± 0.06	0.245 ± 0.04	0.218 ± 0.04	0.223 ± 0.08	0.331 ± 0.06	0.399 ± 0.04	0.245 ± 0.04
0.16	0.308 ± 0.03	0.352 ± 0.04	0.380 ± 0.03	0.350 ± 0.08	0.246 ± 0.06	0.382 ± 0.04	0.439 ± 0.03	0.425 ± 0.08	0.396 ± 0.06
0.2	0.334 ± 0.04	0.418 ± 0.03	0.455 ± 0.05	0.410 ± 0.06	0.360 ± 0.03	0.566 ± 0.03	0.578 ± 0.05	0.452 ± 0.06	0.435 ± 0.03

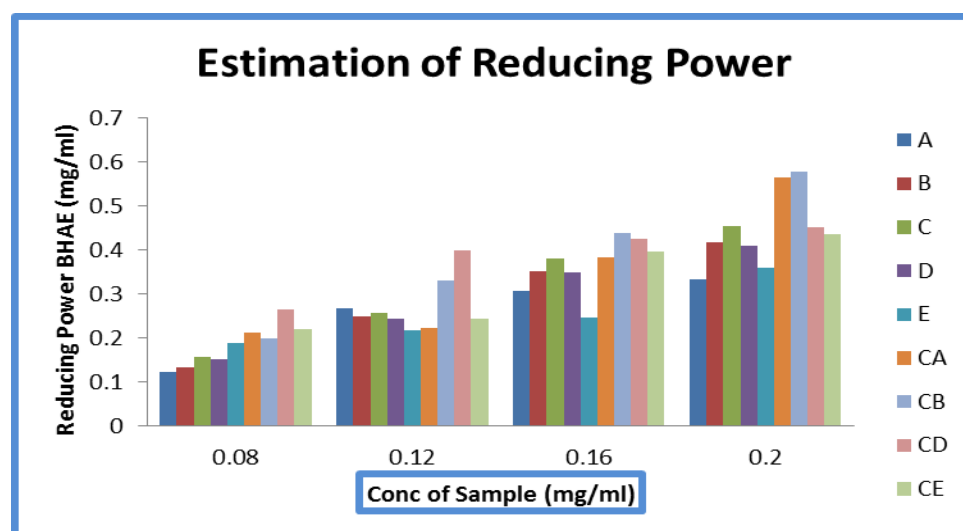


Figure 25: Estimation of Reducing Power Activity. A: Nutgrass (*Cyperus rotundus*), B: Durva (*Cynodon dactylon*) Grass, C: *Vetiveria zizanioides*, D: *Paspalidium flavidum* and E: *Dactyloctenium aegyptium*.

4 Statistical Analysis

Total Phenolic content, Total Flavonoid content and Reducing power assays were performed in triplicate. Mean values for different parameters were calculated and compared by analysis of variance (one-way ANOVA) using online software. Moreover, statistical differences between mean values were identified at confidence level $p < 0.001$.

5 CONCLUSION

It can be concluded that the aerial parts of all the selected species showed antioxidant properties. The maximum antioxidant properties were shown by leaf extract of combination of *Vetiveria zizanioides* and *Cynodon dactylon*. The combination extract has shown more antioxidant activity as compared to individual extract. This may be possible due to the synergetic effect shown by the phenolic or polyphenolic compounds present in the extract. As the samples used in the study are easily found throughout India, they can be considered as a

potential nutraceutical source. The phenols and flavonoids present in the sample can be easily extracted in water and thus offers opportunities to formulate a low cost antioxidant rich food supplement.

6 REFERENCES

1. Kirkinezos IG and Moraes CT 2001 Reactive species and mitochondrial diseases. *Sem. Cell Dev. Biol.*, 12: 449–457.
2. Jena NR and Mishra PC 2005 Mechanisms of formation of 8-oxoguanine due to reactions of one and two OH radicals and the H_2O_2 molecule with guanine: a quantum computational study. *J. Phys. Chem. B*, 109: 14205–14218.
3. Jena NR and Mishra PC 2006 Addition and hydrogen abstraction reactions of an OH radical with 8-oxoguanine. *Chem. Phys. Lett.*, 422: 417–423.
4. Jena NR and Mishra PC 2007 Formation of 8-nitroguanine and 8-oxoguanine due to reaction of

- peroxynitrite with guanine. *J. Comput. Chem.*, 28: 1321–1335.
5. Jena NR and Mishra PC 2012 Formation of ring-opened and rearranged products of guanine: mechanisms and biological significance. *Free Radical Biol. Med.*, 53: 81–94.
 6. Jena NR, Kushwaha PS and Mishra PC 2008 Reaction of hypo- chlorous acid with imidazole: formation of 2-chloro and 2- oxoimidazoles. *J. Comput. Chem.*, 29: 98–107.
 7. Bauer GB and Povirk LF 1997 Specificity and kinetics of inter- strand and intrastrand bifunctional alkylation by nitrogen mustards at a G-G-C sequence. *Nucleic Acid Res.*, 25: 1211–1218.
 8. Bourne, M. (1978). Texture Profile Analysis. *Food Technology*, 32(7): 62–73.
 9. Prior RL, Wu X, Schaich K (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.*, 53: 4290-4302.
 10. Fabricant D S, Farnsworth N R. The value of plants used in traditional medicine for drug discovery. *Environ Health Perspect.*, 2001; 109: 69–75.
 11. Sun, T., Powers, J. R., & Tang, J. Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food Chemistry*, 2007; 105(1): 101–106.
 12. Chavan J.J, Jagpat U.B, Gaikwad N.B, Dixit G.B, Bapat V.A (October-December 2013) Total phenolics, flavonoids and antioxidant activity of Saptarangi (*Salacia chinensis* L.) fruit pulp. *J. Plant Biochem. Biotechnol.*, 22(4): 409-413.
 13. Athukorala, Y., Jeon Y. and Kim, K. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, *Ecklonia cava*. *Food Chemical Toxicology*, 2006; 44(7): 1065-1074.