

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.610.252>

Studies on the Effect of *Ascophyllum nodosum* Extract (ANE) on Growth, Antioxidant Potential and Alpha Glucosidase Inhibition Activity of *Vigna aconitifolia* (RMO 225)

Nidhi Verma¹, Krishan D. Sehrawat² and Anita R. Sehrawat^{1*}

¹Department of Botany, Maharshi Dayanand University, Rohtak-124001, Haryana, India

²Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar, Haryana, India

*Corresponding author

ABSTRACT

Vigna aconitifolia is highly resistant food crop with highly nutritious seed proteins. Among different seaweeds the *Ascophyllum nodosum* is more researched these days, so it is important to study the interaction of the seaweed with different food crops including legumes. We previously found increased tyrosinase inhibition activity in *Vigna aconitifolia* seeds when treated with *Ascophyllum nodosum*. So this study investigated the effects of Biovita, a Seaweed Liquid Fertilizer (SLF) of *Ascophyllum nodosum*, on seed germination, shoot length, root length, fresh weight and dry weight of moth bean seeds. The free radical scavenging by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and alpha glucosidase inhibition activity of treated seeds up to 24 hour (0, 6, 12, 18 and 24 hour) also determined. The seeds of *Vigna aconitifolia* were treated with 0%, 0.01%, 0.05%, 0.10%, 0.50% and 1.0% of *A. nodosum* extract for different time periods ranging from 0 to 24 hours. The seed germination, shoot length, root length, fresh weight and dry weight were found maximum at 0.01% of SLF. The result showed that among different concentration of SLF the 0.05 % was best for free radical scavenging and alpha glucosidase inhibition activity. From the experiment it was observed that with increased time period (0 to 24 hour) free radical scavenging and alpha glucosidase inhibition activity increased but among different concentrations of SLF the activities increased up to 0.05% of the seaweed and decrease thereafter due to toxicity in *Vigna aconitifolia* seeds. The study also showed a positive correlation between radical scavenging and alpha glucosidase inhibition. So seed treatment up to 0.05% was best for seed germination, shoot length, root length, fresh weight, dry weight, free radical scavenging and alpha glucosidase inhibition activity.

Keywords

Vigna aconitifolia,
Ascophyllum nodosum Extract (ANE), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Alpha glucosidase inhibition.

Article Info

Accepted:
21 September 2017
Available Online:
10 October 2017

Introduction

Seaweeds are the macroscopic algae, devoid of roots, stem and leaf and constitute one of the most important marine living resources. On the basis of pigments seaweeds are divided in to three groups, viz., Green (Chlorophyceae), Brown (Phaeophyceae),

Red (Rhodophyceae) algae. The liquid fertilizers that are derived from seaweeds are known as seaweed liquid fertilizers. The fertilizers that are derived from seaweeds are found to be more effective as compared to the chemical fertilizers without any adverse

effects. The natural bioactive materials in seaweeds could be absorbed in plants in a very short time and harmless to the ecosystem (Satya *et al.*, 2010). Seaweeds are rich source of bioactive compounds including hormones, trace elements as well as a potential source of antioxidants and many phytochemical compounds (Ramarajan *et al.*, 2013). Different studies involving different seaweeds on different crops are carried out. For instance, Erulan *et al.*, (2009) showed that the application of the low concentrations of seaweed *Sargassum polycystum* (0.05%) on *Cajanus cajan* increases seed germination, shoot length, root length, fresh weight, and dry weight. Similarly, Kalaiivanan and Venkatesalu (2012) reported increased germination rate and growth rate of *Vigna mungo* in addition to increased amylase activity and sugar content when low concentration of seaweed, *Sargassum myriocystum* were applied. Out of the different groups of seaweeds, the Phaeophyceae (Brown algae) are highly studied groups of sea weeds for their affect on the plants. The liquid extracts of brown seaweeds in India are sold in market under different brand names such as, Renzyme, Alg-A-Mic, Stress-X Powder, and Biovita etc. as biostimulants or biofertilizers.

The effect of brown seaweeds on various leguminous crops showed positive effects. Satya *et al.*, (2010) studied the effect of the application of the three seaweeds viz., *Grateloupia lithophila* (Red algae), *Chaetomorpha linum* (Green Algae), and *Sargassum wightii* (Brown algae) on the photosynthetic pigments and biochemical parameters of *Cajanus cajan*. He found that among these seaweeds at lower concentration *Sargassum wightii* and *Chaetomorpha linum* showed more positive results than *Grateloupia lithophila*. Hernández-Herrera *et al.*, (2016) reported that the brown seaweed, *Padina gymnospora* on tomato and mung

bean plants increase germination percentage, radical and shoot length, and dry weight. Among the brown seaweeds, *Ascophyllum nodosum* is thoroughly studied for its use in agriculture because of its extraordinary capacity to crop plants (Craigie, 2011). The *A. nodosum* contains organic acids, macronutrients, micronutrients and various hormones like cytokinins, auxin, gibberellins, betaines, mannitol and proteins that are important for the crop plant growth (Norrie *et al.*, 1999). The extract of seaweed *Ascophyllum nodosum* (Trade name: Biozyme) increases growth and yield parameters of soybean (Tandon *et al.*, 2015).

Vigna aconitifolia (Jacq.) Marechal, (moth bean/dew bean/matki) is a minor, drought tolerant food crop that belongs to family Fabaceae. *V. aconitifolia* has a high quantity as well as quality of seed proteins and is used as food. Like other pulses, it supplies fair share of proteins to a vegetarian diet. We have studied the effect of *Ascophyllum nodosum* on tyrosinase inhibition activity of *Vigna aconitifolia* (communicated elsewhere). In that study we found increased tyrosinase inhibition activity in *Vigna aconitifolia* sprouts under the influence of *Ascophyllum nodosum*.

Legumes play a very important role in human diet because of major sources of protein, micronutrients, dietary fiber, phytochemicals and low fat content, as well as lower cost as compared to animal proteins (Anderson *et al.*, 1999). Besides being of high nutritional value, legumes also possess therapeutic properties (Geil and Anderson, 1994). The consumption of legumes is directly related to positive health benefits and reducing the risk of various diseases such as, cardiovascular diseases, cancerous diseases, and type-2 diabetes (Messina 1999; Mellen *et al.*, 2008; Cardador-Martinez *et al.*, 2002; McKeown *et al.*, 2002).

Legumes, the main staple food throughout the world, have been more studied towards health benefits related to antioxidants. Antioxidants are the substance that potentially delays or stops oxidation of their substrate (Halliwell *et al.*, 1995). The grains, fruits, pulses and vegetables are the primary sources of natural antioxidants (Anonymous New Delhi, 1988). The antioxidants from the plant based food like vitamin C, vitamin E, carotenes, and phenolic acids have been potentially known to reduce various diseases (Anonymous Mumbai, 2002). The antioxidants scavenge free radicals and reactive oxygen species so as a result inhibit oxidative process that led to various health related disorders (Benzie and Strain, 1996). The synthetic antioxidants create various side effects that are toxic to the human health. Various antioxidants were therefore isolated and purified from various natural sources (Dillard and German, 2000; Wang and Linn, 2000). The natural antioxidants such as ascorbic acid, phenolic compounds, when consumed in diet have been experimentally proved to reduce the risks of chronic diseases ((Pandey and Rizvi, 2009; Hermsdorff *et al.*, 2012). The main causes of these health disorders are due to free radicals and reactive oxygen species (Tepe *et al.*, 2007). The main reason of production of free radical and reactive oxygen species is hectic life style, deep fries, unhealthy food, and environmental pollutants by oxidation process (Turkoglu *et al.*, 2007). The excessive production of free radicals stimulates oxidative process which cause production of more than hundred disorders like cancer, aging, heart disease, inflammatory injuries, central nervous system injury and various neurodegenerative disorders (Tepe *et al.*, 2007; Pong, 2003; Sandhya *et al.*, 2010).

We previously reported (communicated elsewhere) increase in the tyrosinase inhibition activity in *Vigna aconitifolia* seeds when

soaked in *Ascophyllum nodosum*. The extracts with potential anti-tyrosinase are always correlated with strong antioxidant this is because both anti-tyrosinase and antioxidant play an important role in free radical scavenging. The present attempt was made to study the effect of seaweed on germination, Shoot length, Root length, Fresh weight, and Dry weight for ten days. Further, attempts were also made to investigate the effect of different germination time on antioxidant and alpha-glucosidase inhibition activities of *Vigna aconitifolia*.

Materials and Methods

Seaweed, *Ascophyllum nodosum* (trade name: Biovita, PI industries, Udaipur, Rajasthan) and the seeds of *Vigna aconitifolia* (RMO 225) were purchased from Rajasthan, India.

Chemicals: Mercuric chloride, Methanol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, p-nitrophenyl- α -D-glucopyranoside, Sodium carbonate and Sodium phosphate monobasic and dibasic are purchased from Himedia. The enzyme alpha-glucosidase was purchased from Sigma Aldrich.

Seaweed preparation and seed treatment

The seaweed, *Ascophyllum nodosum* extract (ANE) was diluted to 0.01%, 0.05%, 0.10%, 0.50% and 1.0% (v/v) with sterilized water. Only sterile distilled water was taken as negative control. Ten gram of seeds was sterilized with 0.1% of mercuric chloride followed by 2-3 washing with distilled water. The seeds were soaked in 20 ml of different concentrations of seaweed for 12 hours (i.e., 0 hour of sprouting) in a 100ml beaker. After soaking the seaweed was discarded and seeds were left in petriplates containing the different concentrations of seaweed for sprouting for 0, 6, 12, 18 or 24 hours. The

plant extracts were prepared from different time periods (0-24) of all treatments. For germination experiment, the filter paper was placed on the petriplates and wetted with 4 ml of different concentrations of seaweed and for control wetted with sterile distilled water. After 12 hours (i.e., 0 hour) 10 seeds were placed on the filter paper from different concentrations of seaweed and kept under day (fluorescent) light for 10 days.

The temperature of culture room was maintained at 27°C. The germination of seeds were recorded up to 5 days and 10 days old seedlings were taken for observations i.e., shoot length, root length, fresh weight and dry weight. The weight of seedlings was measured before and after drying the seedlings at 65±5°C. The dry weight of seedling was measured till constant weight was observed.

Preparation of plant extracts

One gram of seeds was sprouted for different time periods without or with different concentrations of seaweed and was crushed in 2ml of 80% methanol. The crushed seeds were stirred on an orbital shaker at 200rpm for 10 minutes at room temperature. The crushed material was then placed in water bath for 10 minutes having 60°C temperature and centrifuged it at 10,000 rpm for 10 minutes. The supernatant was taken in a tube and the pellet was again washed with 2ml of 80% methanol. The supernatant at a ratio of 1:4 (w/v) was collected for DPPH assay as well as for the measurement of alpha-glucosidase inhibition activity.

DPPH radical scavenging assay

The antioxidant activities of treated seeds extract was measured in terms of antioxidant that reacts with DPPH radical and reduce them. So the amount of residual DPPH is proportional to absorption at 517nm. The

protocol of Sanchez-Moreno *et al.*, (1998) was used to measure the antioxidant activity with slight modifications. DPPH (6×10^{-5} M) was dissolved in 80% methanol. In a sterile test tube, the DPPH solution (1ml) was mixed with 250µl (250 mg/ml) of plant extracts of different treatment. The reaction mixture was shaken and kept for minimum 30 minutes in the dark at room temperature. The decrease in absorbance was measured at 515nm at the end of incubation period with a Thermo Scientific Evolution 201 UV-visible spectrophotometer. For control equal volume of DPPH and 80% methanol was taken (1:1). Ascorbic acid was used as a positive control. The free radical scavenging activity was calculated using this formula

$$\% \text{ DPPH radical Scavenging} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Alpha-glucosidase inhibition assay (EC 3.2.1.20)

A modified version of protocol described by Shai *et al.*, (2011) was used for analysis of alpha-glucosidase inhibition activity using 4-nitrophenyl α -D-glucopyranoside (PNPG) as substrate. Methanolic seed extract (100 µl, 250mg/ml) of different treatment was mixed with 50 µl of alpha-glucosidase in 0.1M sodium phosphate buffer (1unit/ml) and pre-incubated for 20 minutes at 37 °C. After pre-incubation, 100 µl of p-nitrophenyl- α -D-glucopyranoside (5mM, pH-6.8) was added followed by the addition of 150 µl of sodium carbonate (0.2 M). After this, the absorbance was taken at 405 nm on Thermo Scientific Evolution 201 UV-visible spectrophotometer. For control buffer solution was taken. For positive control, Synthetic anti-diabetic drug acarbose (1mg/ml) was used for the measurement of alpha-glucosidase inhibition activity. The inhibition percentage was calculated using following formula

$$\% \text{ Alpha-Glucosidase inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$$

Statistical analysis

All values were expressed as means \pm standard deviation. The experiment was carried out in triplicates. For germination experiment the sample size (n) was 30 for germination percentage, shoot length and root length and n was 3 for fresh weight, dry weight, DPPH and alpha glucosidase inhibition activities. The differences obtained in all the above parameters were subjected to the test of significance using one way analysis of variance (ANOVA). Further, the differences between the groups were tested by employing HSD Tukey test. P values ≤ 0.05 were considered as significant.

Results and Discussion

The morphological changes in seeds after treatment with different concentrations of *Ascophyllum nodosum* extract (ANE) are shown in Figure 1.

Growth parameters

The effect of different concentrations of *Ascophyllum nodosum* extract (ANE) on *Vigna aconitifolia* was studied. The parameters such as seed germination, shoot length, root length, fresh and dry weights were recorded.

Seed germination

The maximum germination of seeds took place on the 3rd day (ANOVA; F= 23.32; $p < 0.0001$ HSD Tukey; p value < 0.01). The germination percentage ranged from 0 to 1% of treatment (66.67 \pm 15.28 to 3.33 \pm 5.77). The treatment of different concentration of ANE

in seeds of *Vigna aconitifolia*, the germination percentage was decreased with the increase in concentration. The percentage of seed germination was increased up to 0.05% of treatment thereafter germination percentage was decreased. The maximum germination percentage was obtained at 0.01% (96.67 \pm 5.77) of ANE treatment. There was almost no germination in seeds upon 1.0% of ANE treatment (Fig. 2). Our result coincides with the findings of Arun *et al.*, (2014) who showed better germination in seeds of *Abelmoschus esculentus* and *Solanum lycopersicum* at middle concentrations (40 to 60 %) of seaweeds. Thirumaran *et al.*, (2009) found maximum seed germination in *Abelmoschus esculentus* when treated with 20 % *Rosenvigea intricata* Seaweed Liquid Fertilizer. Kalaivanan and Venkatesalu, (2012) studying the effect of brown seaweed *Sargassum polycystum* on germination and growth of *Vigna mungo*. They soaked *Vigna mungo* seeds in different concentration of SLF *Sargassum myriocystum* (5%, 10%, 25%, 50% and 75%) for 12 hours and found maximum seed germination at 10% of SLF.

Shoot length and root length

The shoot length and root length (cm) of germinated seedlings were measured on 10th day after treatment with different concentration of ANE. The maximum shoot length (4.67 cm) was obtained upon 0.01% of treatment (ANOVA; F=5.31; $p = 0.00183$). The minimum or no shoot length was obtained at the highest concentration (i.e., 0.5 and 1.0%) of ANE treatment (Fig. 3). The longest root length was also recorded in 0.01% of treated seaweed concentration (ANOVA; F=20.20; $p < 0.0001$). In the present study the seedlings treated with low concentration (0.01%) of SLF showed better response of shoot length and root length. Erulan *et al.*, (2009) found the *Cajanus cajan* had highest shoot length and root length after

lower concentration treatment of SLF *Sargassum polycystum*. Other studies also indicated that lower or optimum concentration of different SLF increases shoot length and root length in different crops (John and Yuvaraj, 2014; Ramya *et al.*, 2012; Kavipriya *et al.*, 2011; Jothinayagi *et al.*, 2009).

Fresh weight and dry weight

Fresh weight of ANE treated seedlings ranged from 6.24±0.40 to 2.09±0.070 fresh weight of seedlings and maximum value was found at 0.01 % concentration of ANE treatment. The lowest value obtained at 1.0% of treatment.

The fresh weight increased up to 0.05% concentration of treatment there after it showed decline manner. The dry weight value ranges from 2.25±0.03to 0.6±0.06 (ANOVA; F=99.6; p<0.0001). The maximum and minimum values of dry weight found at 0.01 % and 1.0% of respectively. The figure is plotted between shoot length and root length of germinated seedlings at different concentrations (Fig. 4). The water content and moisture content of treated seedlings was also measured (Fig. 4). The maximum values of water content and moisture content were 3.99±0.4 and 67.64±0.96 at concentration of 0.01% and 0.05% of treatment respectively.

Fig.1 Seed of *Vigna aconitifolia* (RMO 225) showing germination at different concentrations of ANE on 7th day of germination

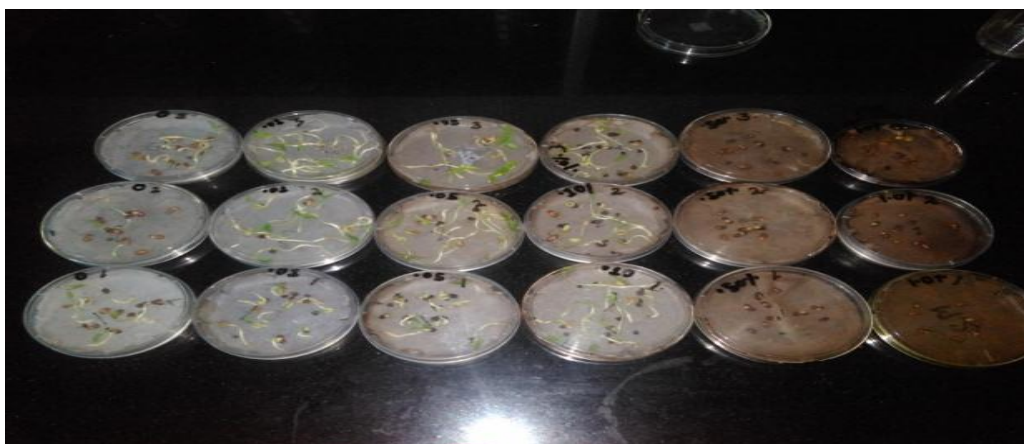


Fig.2 Graph showing germination of seed under the influence of ANE on 3rd day of their germination

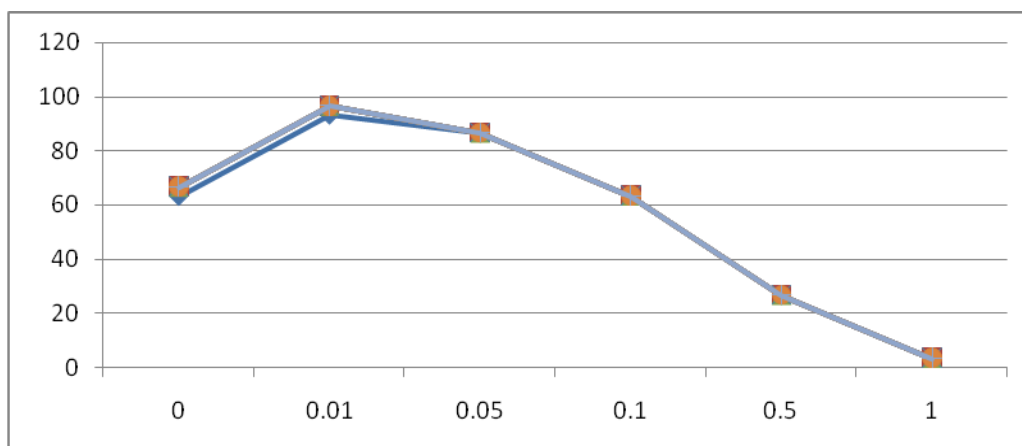


Fig.3 Graphs showing that ANE (0.01%) was the best for growth of seedlings on 10th day of germination

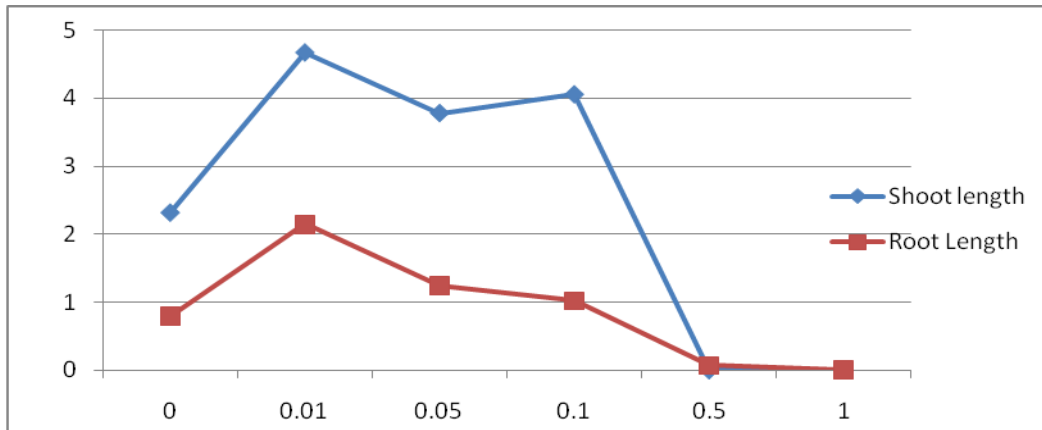


Fig.4 Showing organic content, water content and percent moisture in seedling at different ANE (0.01%)

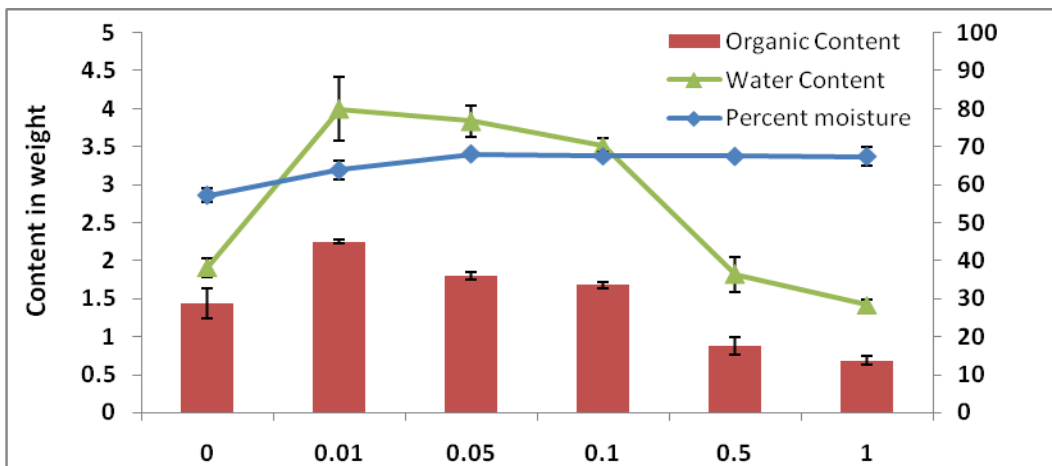


Fig.5 Antioxidant potential of *Vigna aconitifolia* at different time intervals (0-24Hrs.) under the influence of different conc. of ANE

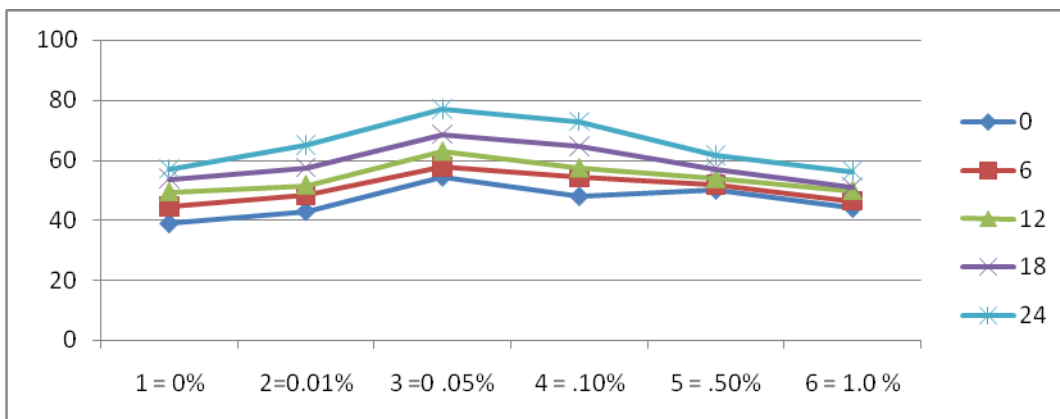
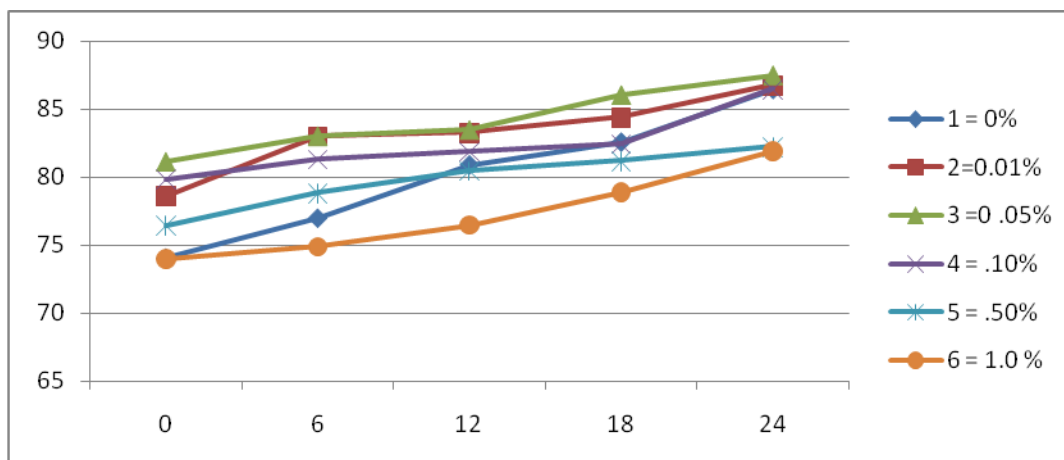


Fig.6 Effect of different concentration of ANE on Alpha glucosidase inhibition activity of *Vigna aconitifolia* at different time intervals (0-24Hrs.)



The obtained results were coinciding with the previous studies of Kavipriya *et al.*, (2011) found, lower concentration of SLFs increases the organic matter in *Vigna radiata* seedlings. Kalaivanan and Venkatesalu (2012) also recorded maximum fresh weight and dry weight in *Vigna mungo* seedlings at 10% of *S. myriocystum* treatment.

DPPH radical scavenging activity

Antioxidants are the main defensive part that provide resistant to oxidative stress caused by free radicals. Prior to germination, when *V. aconitifolia* seeds were soaked in the seaweed for 12 hours and the tyrosinase inhibition was calculated, the results showed maximum activity at 24 hour of germination as compared to the respective control (communicated elsewhere). The findings may be suggestive of the importance of investigating the antioxidant potential of seaweed on the seeds of *V. aconitifolia* because both of these activities are based on free radicals. Hence, the *V. aconitifolia* seeds were soaked in the seaweed for 12 hours and the DPPH radical scavenging activity was recorded in the subsequent 24 hours at a regular time interval of 6 hours each (Fig. 5). The DPPH activity was found to be maximum

at 24 hour of sprouting (ANOVA; $F= 12246$; $p<0.0001$) as compared to the 0 hour (ANOVA; $F= 733.31$; $p<0.0001$). The free radical scavenging activity of treated *V. aconitifolia* seeds increased from 0 to 24 hour (HSD Tukey; p value <0.01). Among different concentration of seaweed treatment (0.0 to 1.0%) the maximum antioxidant potential was found at 0.05% of seaweed concentration (ANOVA; $F=203.37$; $p<0.0001$) as compared to control (ANOVA; $F= 660.87$; $p<0.0001$). Coincidentally, the tyrosinase inhibition activity observed in treated seeds of *A. nodosum* was maximal at 24 hour in case of treatment with the 0.05% seaweed. Legumes are attracting attention of the workers around the world due to its beneficial properties in relation to the human health. Legumes play very important role in prevention of cellular and molecular damage by decreasing free radicals. Legume germination is an effective process that increases their antioxidant potential (Fernandez-Orozco *et al.*, 2006). Seaweed extracts contains high level of phenolic compounds and shows many antioxidant properties (Tong *et al.*, 2014). *Ascophyllum nodosum* is high in phlorotannins which act as potent antioxidant comparable to Quercetin or Trolox (Blanc *et al.*, 2011). The treatment of commercial extract of *Ascophyllum nodosum*

increases antioxidant activity of *Spanacia oleracia*, and also the flavonoid and total phenolic content (Fan *et al.*, 2012). Kim *et al.*, (2012) found highest DPPH activity in mung bean sprouts than seeds. The antioxidant activity of mung bean sprouts was 6 times higher than seeds reported by Guo *et al.*, (2012). Zhaohui *et al.*, (2016) reported increased antioxidant activity in germinated mung bean, soybean and black bean sprouts of 3-5 days. Gan *et al.*, (2016) found increased in ascorbic acid, total phenolic content and antioxidant capacity in mung beans up on germination. Marathe *et al.*, (2012) showed potential antioxidant activity of moth bean. Our results coincides with that of Kestwal *et al.*, (2012), who showed at cellular level significantly increase in nutrient content and antioxidant potential of germinated moth bean seeds.

The treated germinated seeds showed potential free radical scavenging activity against synthetic DPPH which may be due to hydrogen donating capacity. So this ability showed or claimed to prevent human health related many diseases like diabetes, cancer, ageing, atherosclerosis etc that are associated with free radicals.

Alpha-glucosidase inhibition activity

Alpha-Glucosidase is secreted by the cells that lines small intestine, is the one of the most important enzyme for the breakdown of carbohydrates in to glucose and its absorption in the intestine results hyperglycemia. Alpha-Glucosidase inhibitor control hyperglycemia by delays glucose absorption through competitive and inhibition of intestinal α -glucosidase enzyme (Vadiveland Biesalski, 2012; Gwtek *et al.*, 2014). *Ascophyllum nodosum* contains compounds called ascophyllan and fucoiden, which is investigated for its immune-stimulatory properties that inhibit carbohydrate absorption

(Zhang *et al.*, 2007; Kim *et al.*, 2014). Carbohydrate inhibition is related to polyphenolic content. The polyphenolic compounds of *Ascophyllum nodosum* are known to act on α -glucosidase inhibitory potential (Apostolidis *et al.*, 2011). Various types of synthetic antidiabetic drugs are sold in the market to reduce hyperglycemia but these cause various side effects to human health. Paradis *et al.*, (2011) found the two brown seaweeds (*Ascophyllum nodosum* and *Fucus vesiculosus*) potentially inhibit α -amylase and α -glucosidase without any adverse effect. In mice Zhang *et al.*, (2007) found the aqueous ethanolic extract of *Ascophyllum nodosum* inhibiting rat intestinal α -glucosidase ($IC_{50} = 77 \mu\text{g/mL}$) as compared to untreated diabetic mice. The *Ascophyllum nodosum* preparation also improved blood antioxidant activity. Previous studies showed a positive relationship between phenolic content, antioxidant capacity and alpha-glucosidase inhibitory activity of edible plants reported by Mai *et al.*, 2007.

Because there was a positive correlation between antioxidant potential and alpha-glucosidase inhibition, the experiment was conducted to check alpha-glucosidase inhibition activity of the ANE treated moth bean seeds. The maximum alpha-glucosidase inhibition activity was found at 24 hour (Fig. 6) of sprouting (ANOVA; $F= 3600.53$; $p<0.0001$). Like DPPH activity the alpha-glucosidase inhibition activity was also increased from 0 to 24 hour of sprouting (HSD Tukey; $p \text{ value}<0.01$). Among all concentrations 0.05 % of ANE concentration was found to be very effective for inhibition of alpha-glucosidase (ANOVA; $F= 5191.5$; $p<0.0001$). The maximum alpha-glucosidase inhibition value of moth bean seeds at 24 hour of 0.05% was 87.49 ± 0.04 , this value was found maximum than synthetic antidiabetic drug, acarbose. Our results coincides with previous studies of Yao *et al.*, (2010) who

shows significant positive correlation of the antioxidant activity and alpha-glucosidase inhibitory activity of coloured grains in china. Sreerama *et al.*, (2012) showed the IC₅₀ alpha-glucosidase value of moth bean extracts 50.42 mg/mL. Burguieres *et al.*, (2006) reported in pea upon germination increased phenolic-enriched content was more effective to control alpha-glucosidase in relation to hyperglycemia. The results of our study showed that with increased time period's DPPH as well as alpha-glucosidase inhibitory activity increased. Among different concentrations of *Ascophyllum nodosum*, the 0.01 % was the best for seed germination, shoot length, root length, fresh weight, dry weight and 0.05 % concentration was the best for both free radical scavenging and reduction of hyperglycemia. A significant correlation was found among all activities. Significant contribution was found from ANE treated seeds for antioxidant and enzyme inhibition activities. To the best of our knowledge, this is the first study reporting DPPH and alpha-glucosidase inhibition of *Ascophyllum nodosum* treated *Vigna aconitifolia* seeds. The data suggest further isolation and identification of phytochemicals from treated seeds and their bioactive capacities to understand the underlying mechanism of these activities.

For the first time antioxidant and antidiabetic potential of *Vigna aconitifolia* seeds with *Ascophyllum nodosum* at different time periods were studied. Methanolic extracts of all treated seeds shows both activities low to high. It was observed that seaweed extract at low concentration give better results of seed germination, shoot length, root length, fresh weight, dry weight, free radical scavenging and alpha glucosidase inhibition activity. The increase in all above activities in moth bean seeds by SLF may be due to presence of bioactive constituents in *Ascophyllum nodosum*.

Acknowledgment

The authors wish to thank to the Department of Botany, M. D. University, Rohtak for providing all the necessary facilities and other support during the present research.

Conflict of Interest

The authors declare that they have no conflict of Interest

References

- Anderson, J. W., Smith, B. M., and Washnock, C. S. 1999. Cardio-vascular and renal benefits of dry bean and soybean intake. *American Journal of Clinical Nutrition*. 70: 464–474
- Anonymous, Indian herbal pharmacopoeia, revised new edn, Indian drug manufacturers association, Mumbai, 2002, 79-87
- Anonymous, The Wealth of india- A dictionary of Indian raw materials and industrial products, revised edn, Publication and Information Directorate, CSIR, New delhi, 1988, Vol-II B, 119-120
- Apostolidis, E., Lee, and C.M. 2010. *In vitro* potential of *Ascophyllum nodosum* phenolic antioxidant mediated alpha-glucosidase and alpha-amylase. *Journal of Food Science*. 75: 97–102 doi:10.1111/j.1750-3841.2010.01544.x.
- Arun, D., Gayathri, P. K., Chandran, M. and Yuvaraj, D. 2014. Studies on effect of seaweed extracts on crop plants and microbes. *International Journal of ChemTech Research*. 6(9): 4235–4240
- Benzie, I. F., Strain, J. J. 1999. Ferric reducing/antioxidant power assay direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid

- concentration. *Methods Enzymol.* 299: 15-27
- Blanc, N., Hauchard D., Audibert L., and Ar Gall E. 2011. A Radical-scavenging capacity of phenol fractions in the brown seaweed *Ascophyllum nodosum*: an electrochemical approach. *Talanta.* 84(2): 513–518
- Burguieres, E., Mccue, P., Kwon, Y.I., and Shetty, K. 2008. Health-related functionality of phenolic-enriched pea sprouts in relation to diabetes and hypertension management. *Food Biochemistry.* 32: 3–14
- Cardador-Martinez, A., Loarca-pina, G., and Oomah, B.D., 2002. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). *Journal of Agriculture and Food Chemistry.* 50: 6975–6980
- Craigie, J. S., 2011. Seaweed extract stimuli in plant science and agriculture. *Journal of Applied Phycology.* 23(3): 371–393 doi: 10.1007/s10811-010-9560-4.
- Dillard, C.D., and J.B. German. 2000. Phytochemicals: Nutraceuticals and human health. *J. Sci. Food Agric.* 80: 1744-1756
- Erulan, V., Soundarapandian, P., Thirumaran G., and Ananthan, G. 2009. Studies on the effect of *Sargassum polycystum* extract on the growth and biochemical composition of *Cajanus cajan* (L.) Mill sp. *American-eurasian j. Agric. and environ. Sci.* 6 (4): 392-399
- Erulan, V., Soundarapandian, P., Thirumaran, G and Ananthan. 2009. Studies on the effect of *Sargassum polycystum* extract on the growth and biochemical composition of *Cajanus cajan* (L) mil sp. *American- Eurasian J. Agric and Environ. Sci.* 6(4): 392-399
- Fan, D., Hodges, D.M., Zhang, J., Kirby, C.W., Ji, X., Locke, S.J., Critchley A.T., and Prithiviraj B. 2011. Commercial extract of the brown seaweed *Ascophyllum nodosum* enhances phenolic antioxidant content of spinach (*Spinacia oleracea* L.) which protects *Caenorhabditis elegans* against oxidative and thermal stress. *Food chemistry.* 124(1): 195-202
- Fernandez-Orozco, R., Piskula, M.K., Zielinski, H., Kozłowska, H., Frias, J., Vidal-Valverde, C. 2006 Germination as a process to improve the antioxidant capacity of *Lupinus angustifolius* L. var. Zapaton. *European Food Research and Technology.* 223: 495–502
- Gan, R.Y., Wang, M.F., Lui, W.Y., Wu, K., and Corke, H. 2016. Dynamic changes in phytochemical composition and antioxidant capacity in green and black mung bean (*Vigna radiata*) sprouts. *International Journal of Food Science and Technology.* 51: 2090–2098. doi:10.1111/ijfs.13185
- Geil, P.B., and Anderson, J.W. 1994. Nutrition and health implications of dry beans: a review. *Journal of the American College of Nutrition.* 13: 549–558.
- Guo, X., Li, T., Tang, K., and Liu, R.H. 2012. Effect of germination on phytochemical profiles and antioxidant activity of mung bean sprouts (*Vigna radiata*). *Journal of Agricultural and Food Chemistry.* 60: 11050–11055.
- Gwtek, M., Natalia, C., MaBgorzataMuc-Wierzgo, N., Grochowska-Niedworok, E., Kokot, T., Nowakowska-Zajdel, E. 2014. The Active Role of Leguminous Plant Components in Type 2 Diabetes. *Hindawi Publishing Corporation.* 1: 1-12 doi:10.1155/2014/293961
- Halliwel, B., Aeschbach, R., Ltliger, J. and Aruoma, O. I. 1995. The characterization of antioxidants. *Food and Chemical Toxicology.* 33: 601-617
- Hermisdorff, H.H.M., Barbosa, K.B.F., and Volp, A.C.P. (2012). Vitamin C and fibre consumption from fruits and vegetables improves oxidative stress

- markers in healthy young adults. *British Journal of Nutrition*. 107: 1119–1127
- Hernandez-Herrera, R. M., Santacruz-Ruvalcaba, F., Zanudo-Hernandez, J. and Hernandez-Carmona, G. 2016. Activity of seaweed extracts and polysaccharide-enriched extracts from *Ulva lactuca* and *Padina gymnospora* as growth promoters of tomato and mung bean plants. *Journal of Applied Phycology*. 28(4): 2549–2560 doi: 10.1007/s10811-015-0781-4.
- John Peter Paul, J., and Yuvaraj, P. 2014. Effect of Seaweed Liquid Fertilizer of *Colpomenia sinuosa* (Mert. ex Roth) Derbes and Solier (Brown Seaweed) on *Vigna radiata* (L.) R. Wilczek. In Koothankuzhi, Tirunelveli district, Tamil Nadu, India. *International journal of pure and applied bioscience*. 2(3):177–184
- Jothinayagi, N., and Anbazhagan, C. 2009. Effect of Seaweed Liquid Fertilizer of *Sargassum Wightii* on the Growth and Biochemical Characteristics of *Abelmoschus esculentus*. *Recent Research in Science and Technology*. 1(4): 155–158
- Kalaivanan, C., and Venkatesalu, V. 2012. Utilization of seaweed *Sargassum myriocystum* extracts as a stimulant of seedlings of *Vigna mungo* (L.) Hepper. *Span J Agr Res*. 10(2): 466–470 doi: 10.5424/sjar/2012102-507-10.
- Kavipriya, R., Dhanalakshmi, P. K., Jayashree, S. and Thangaraju, N. 2011. Seaweed extract as a biostimulant for legume crop, green gram. *Journal of Ecobiotechnology*. 3(8): 16–19
- Kestwal, R. M., Bagal-Kestwal, D., and Chiang, B.H. 2011. 1,3- β -Glucanase from *Vigna aconitifolia* and its possible use in enzyme bioreactor fabrication. *Int. J. Biol. Macromol*. 49: 894–899
- Kim, D. K., Jeong, S. C., Gorinstein, S. and Chon, S. U. 2012. Total Polyphenols, Antioxidant and Antiproliferative Activities of Different Extracts in Mungbean Seeds and Sprouts. *Plant Foods for Human Nutrition*. 67: 71–75 DOI doi: 10.1007/s11130-011-0273-x.
- Kim, K.T., Rioux, L.E., and Turgeon S.L. (2014). Alpha-amylase and alpha-glucosidase inhibition is differentially modulated by *fucoidan* obtained from *Fucus vesiculosus* and *Ascophyllum nodosum*. *Phytochemistry*. 98: 27-33
- Mai, T.T., Thu, N.N., Tien, P.G., and Van Chuyen, N. 2007. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of nutritional science and vitaminology*. 53: 267–276 doi:10.3177/jnsv.53.267
- Marathe, S.A., Rajalakshmi, V., Jamdar, S.N., and Sharma, A. 2012. Comparative study on antioxidant activity of different varieties of commonly consumed legumes in India, *Food Chem Toxic*. 49: 2005–2012
- McKeown, N. M., Meigs, J. B., Liu, S., and Wilson, P. W.F. Jacques, P. F. 2002. Whole-grain intake is favorably associated with metabolic risk factors for type 2 diabetes and cardiovascular disease in the Framingham Offspring Study. *American Journal of Clinical Nutrition*. 76: 390-398.
- Mellen, P. B., Walsh, T. F., and Herrington, D. M. 2008. Whole grain intake and cardiovascular disease: a meta-analysis. *Nutrition, Metabolism and Cardiovascular Diseases*. 18: 283-290.
- Messina, M.J., 1999. Legumes and soybeans: overview of their nutritional profiles and health effects. *American Journal of Clinical Nutrition*. 70: 439–450
- Norrie, J., Hiltz DA 1999. Seaweed extract research and applications in agriculture. *Agro Food Ind Hi-Tech* 10:15–18

- Pandey, K.B., and Rizvi, S.I. 2009. Plant polyphenols as dietary antioxidants in human health and disease. *Oxidative Medicine and Cellular Longevity*. 2: 270–278
- Paradis, M.E., Couture, P., and Lamarche, B. 2011. A randomised crossover placebo-controlled trial investigating the effect of brown seaweed (*Ascophyllum nodosum* and *Fucus vesiculosus*) on postchallenge plasma glucose and insulin levels in men and women. *Appl Physiol Nutr Metab*. 36: 913–919 doi:10.1139/h11-115
- Pong, K., 2003. Oxidative stress in neurodegenerative disease: Therapeutic implication for superoxide dismutase mimetics. *Exp. Opin. Biol. Ther.* 3: 127–139
- Ramarajan, S., H. J. L. S. G. A. 2013. Effect of Seaweed Extracts Mediated Changes in Leaf Area and Pigment Concentration in Soybean under Salt Stress Condition. *Research and Reviews: A Journal of Life Sciences*. 3(1):17–21
- Ramya, S. S., Vijayanand, N. and Rathinavel, S. 2012. Influence of Seaweed Liquid fertilizers on growth, biochemical and yield parameters of Cluster bean plant. *J. Green Bioenergy*. 1(1): 19–32
- Sanchez-Moreno, C., Larrauri, J.I., and Saura-Calixto, F.A. 1998. A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*. 76: 270–276
- Sandhya, B., Manoharan, S., Sirisha Lavanya, G., Manmohan, C.R. 2010. Lipid peroxidation and antioxidant status in prostate cancer patients. *Indian J. Sci. Technol*. 3(1): 83-86
- Sathya, B., Indu. H., Seenivasan R., and Geetha. S. 2010. Influence of Seaweed Liquid Fertilizer on the Growth and Biochemical Composition of Legume Crop, *Cajanus cajan* (L.) Mill sp. *Journal of Phytology*. 2(5): 50–63
- Shai, L.J., Magano, S.R., Lebelo, S.L., Mogale, A.M. 2011. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: Comparison with their effects on yeast alpha-glucosidase. *J Med Plant Res*. 5:2863–67.
- Sreerama, Y. N., Takahashi, Y. and Yamaki, K. 2012. Phenolic Antioxidants in Some Vigna Species of Legumes and their Distinct Inhibitory Effects on ??-Glucosidase and Pancreatic Lipase Activities. *Journal of Food Science*. 77: 927-933 doi: 10.1111/j.1750-3841.2012.02848.x.
- Tandon, S., and Dubey, A. 2015. Effects of Biozyme (*Ascophyllum nodosum*) Biostimulant on Growth and Development of Soybean [*Glycine Max* (L.) Merrill]. *Communications in Soil Science and Plant Analysis*. 46(7): 845–858 doi: 10.1080/00103624.2015.1011749.
- Tepe, B., Eminagaoglu, O., Akpulat, H.A., and Aydin, E. 2007. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia verticillata* (L.) subsp. verticillata and *S. verticillata* (L.) subsp. amasiaca (Freyn and Bornm.) Bornm. *Food Chemistry*. 100: 985–989
- Thirumaran, G., Arumugam, M., Arumugam, R. and Anantharaman, P. 2009. Effect of Seaweed Liquid Fertilizer on Growth and Pigment Concentration of *Abelmoschus esculentus* (l) medikus. *American-Eurasian Journal of Agronomy*. 2(2): 57–66
- Tong, T., Li, J., Ko, D. O., Kim, B. S., Zhang, C., Ham, K. S. and Kang, S. G. 2014. In vitro antioxidant potential and inhibitory effect of seaweed on enzymes relevant for hyperglycemia. *Food Science and Biotechnology*. 23(6):

- 2037-2044 doi: 10.1007/s10068-014-0277-z.
- Turkoglu, A., Duru, M.E., Mercan, N., Kivrak, I., and Gezer, K. 2007. Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chemistry*. 101: 267–273
- Vadivel, V., and Biesalski, H.K. 2012. Total phenolic content, *in vitro* antioxidant activity and type II diabetes relevant enzyme inhibition properties of methanolic extract of traditionally processed underutilized food legume, *Acacia nilotica* (L.) Willd ex. Delile. *International Food Research Journal*. 19(2):593-601
- Wang, S.Y., and H.S. Linn. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *J. Sci. Food Agric*. 48: 140-146
- Yao, Y., Sang, W., Zhou, M., and Ren, G. 2010. Antioxidant and α -glucosidase inhibitory activity of colored grains in China. *Journal of Agricultural and Food Chemistry*. 58: 770–774 doi: 10.1021/jf903234c.
- Zhang, J., Tiller, C., Shen, J., Wang, C., Girouard, G.S., Dennis, D., Barrow, C.J., Miao, M., and Ewart, H.S. 2007. Antidiabetic properties of polysaccharide- and polyphenolic-enriched fractions from the brown seaweed *Ascophyllum nodosum*. *Canadian Journal of Physiology and Pharmacology*. 85: 1116–1123 doi:10.1139/Y07-099
- Zhang, J., Tiller, C., Shen, J., Wang, C., Girouard, G.S., Dennis, D., Barrow, C.J., Miao, M., and Ewart, H.S. 2007. Antidiabetic properties of polysaccharide- and polyphenolic-enriched fractions from the brown seaweed *Ascophyllum nodosum*. *Canadian Journal of Physiology and Pharmacology*. 85:1116–1123 doi: 10.1139/Y07-099
- Zhaohui, X., Cen Wan, G., Lijuan, Z., Wancong, Y., Huiru C., Xiaohong, K., and Fengjuan, Z. 2016. Bioactive Compounds and Antioxidant Activity of Mung Bean (*Vigna radiata* L.), Soybean (*Glycine max* L.) and Black Bean (*Phaseolus vulgaris* L.) during the Germination Process. *Czech J. Food Sci*. 34: 68–78. doi: 10.17221/434/2015-CJFS

How to cite this article:

Nidhi Verma, Krishan D. Sehrawat and Anita R. Sehrawat. 2017. Studies on the Effect of *Ascophyllum nodosum* Extract (ANE) on Growth, Antioxidant Potential and Alpha Glucosidase Inhibition Activity of *Vigna aconitifolia* (RMO 225). *Int.J.Curr.Microbiol.App.Sci*. 6(10): 2125-2138. doi: <https://doi.org/10.20546/ijcmas.2017.610.252>